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(54) SELF-ASSEMBLING PROTEIN
NANOSTRUCTURES DISPLAYING
PARAMYXOVIRUS AND/OR PNEUMOVIRUS
F PROTEINS AND THEIR USE

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ABSTRACT

Disclosed herein are nanostructures and their use, where the nanostructures include

- (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.

Specification includes a Sequence Listing.

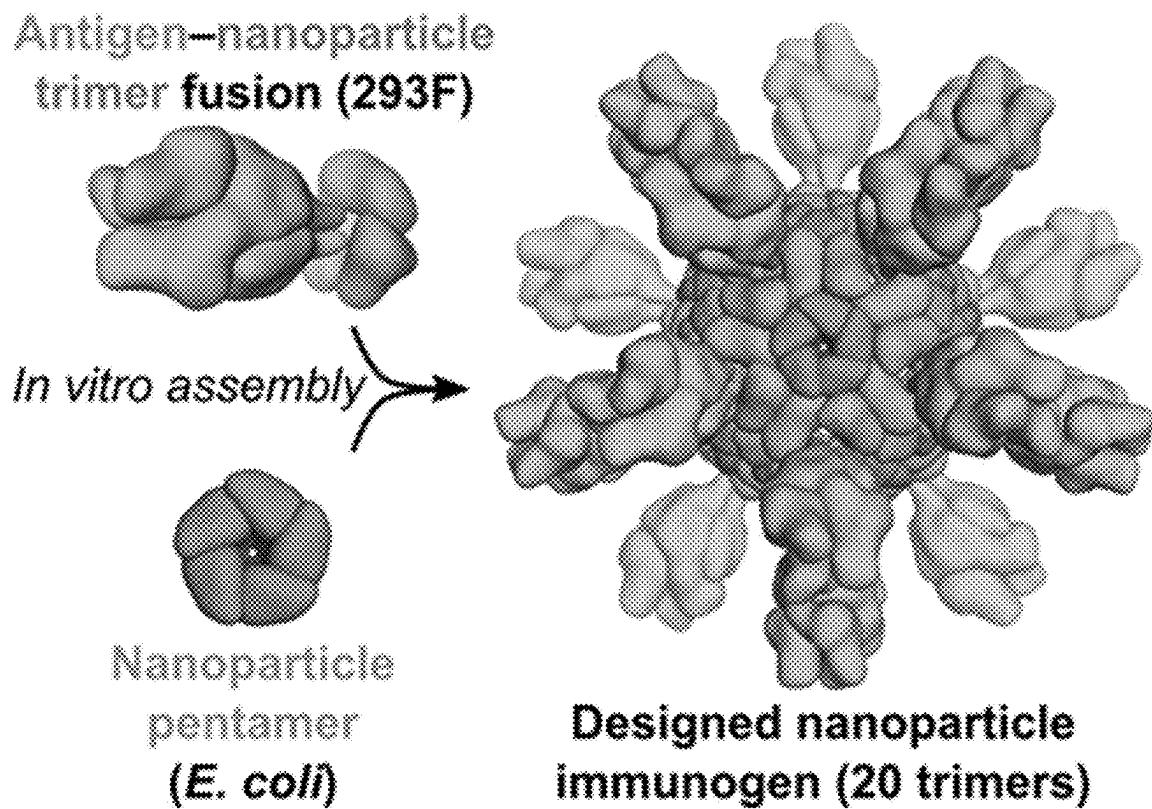


FIG. 1

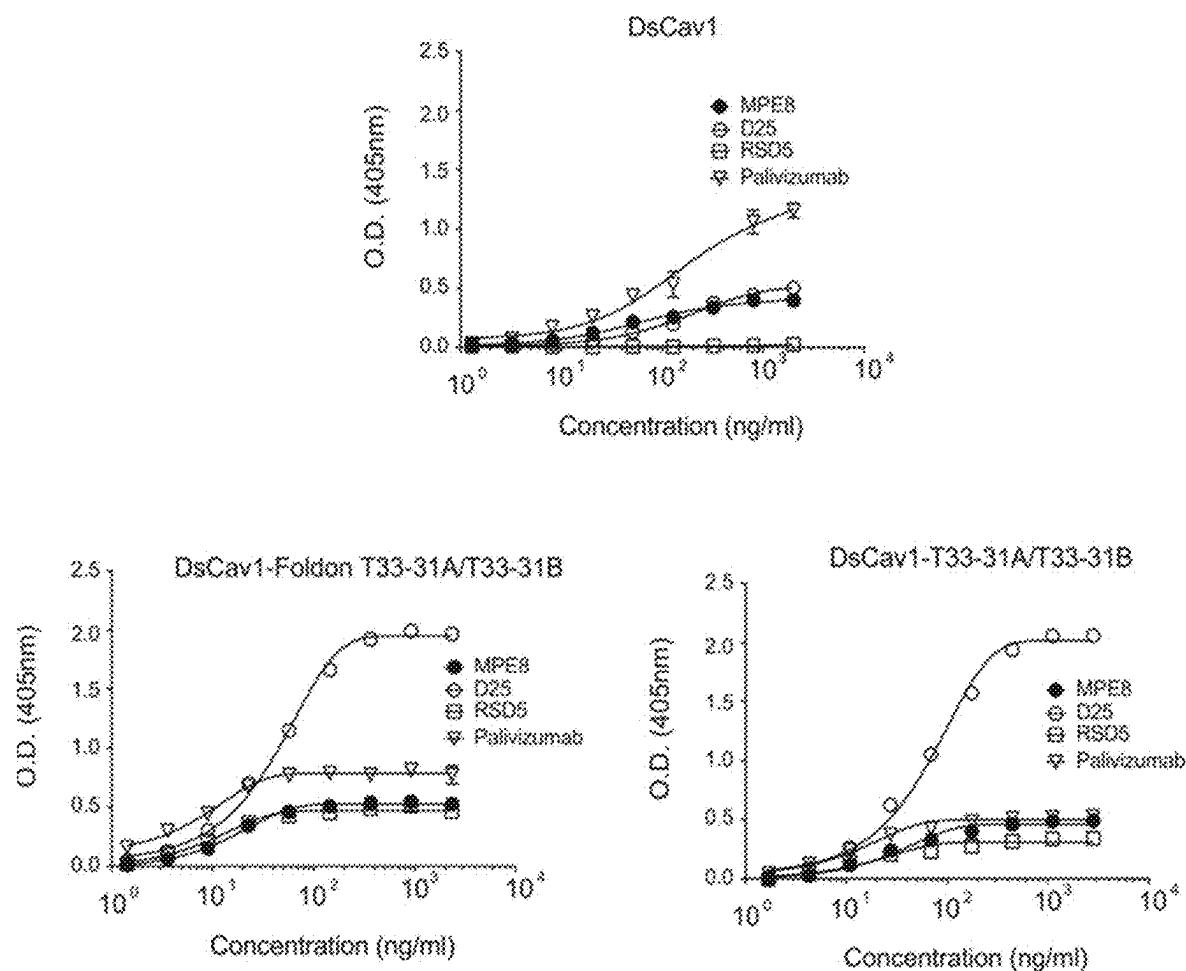


FIG. 2

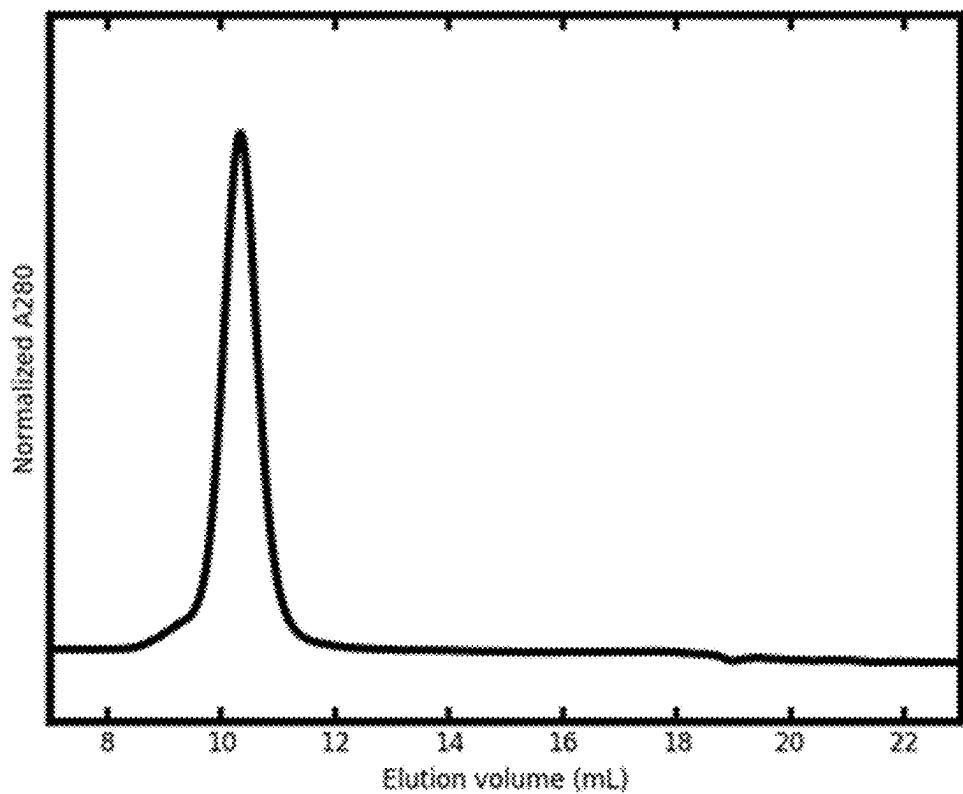


FIG. 3

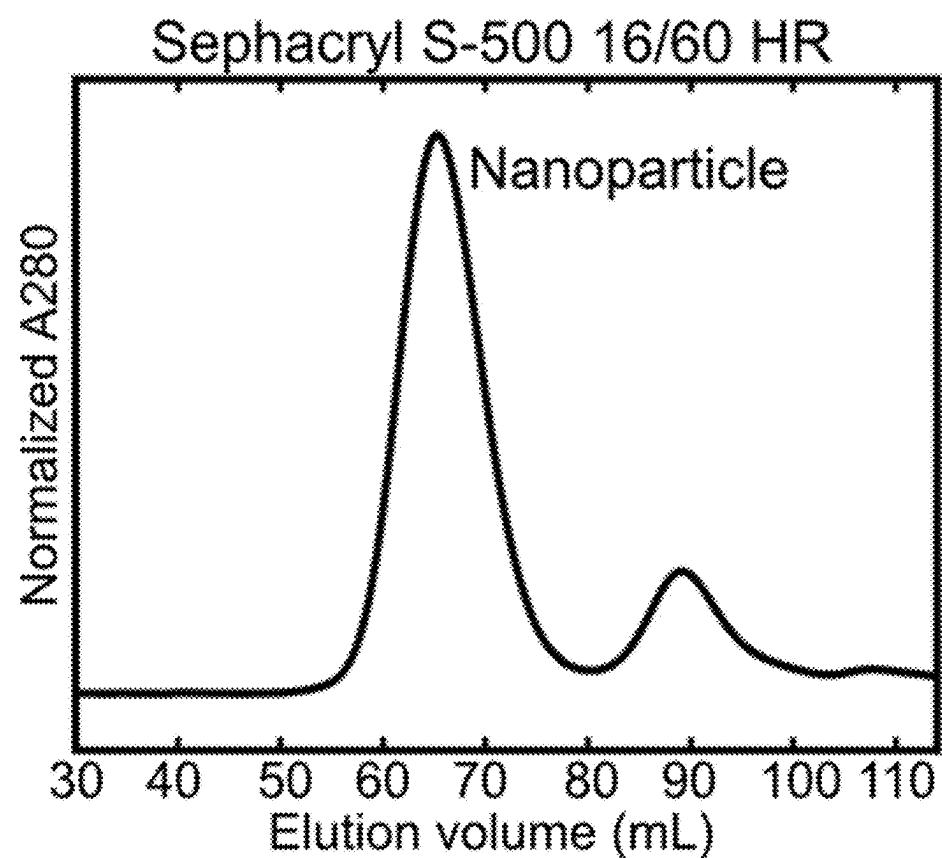


FIG. 4

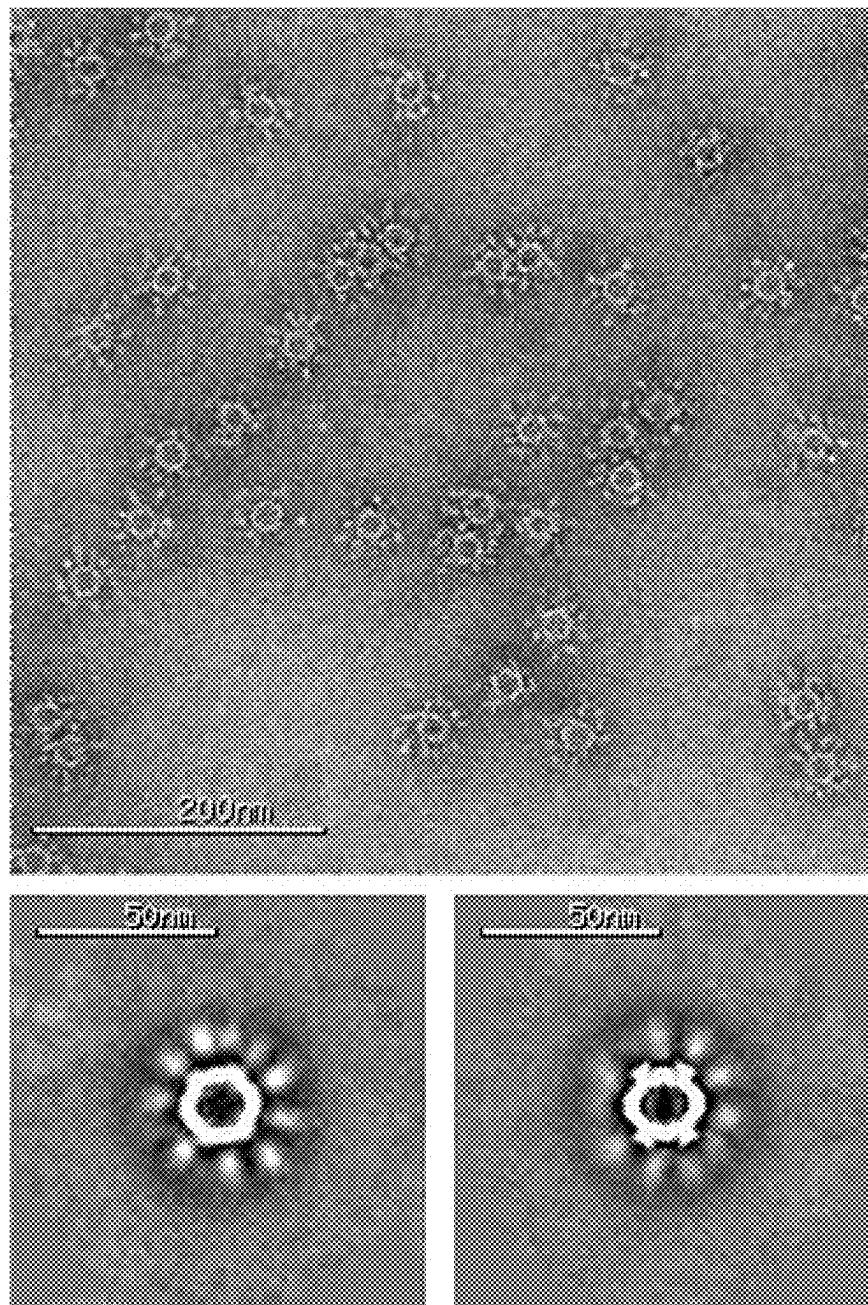


FIG. 5

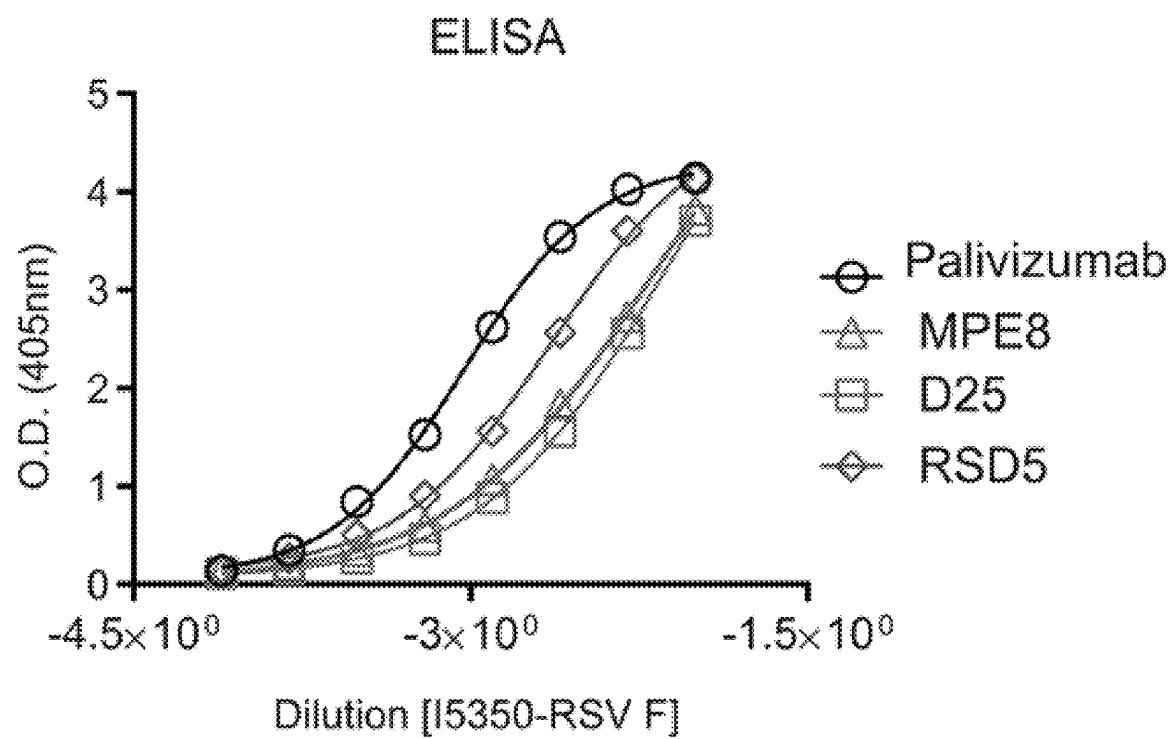


FIG. 6A

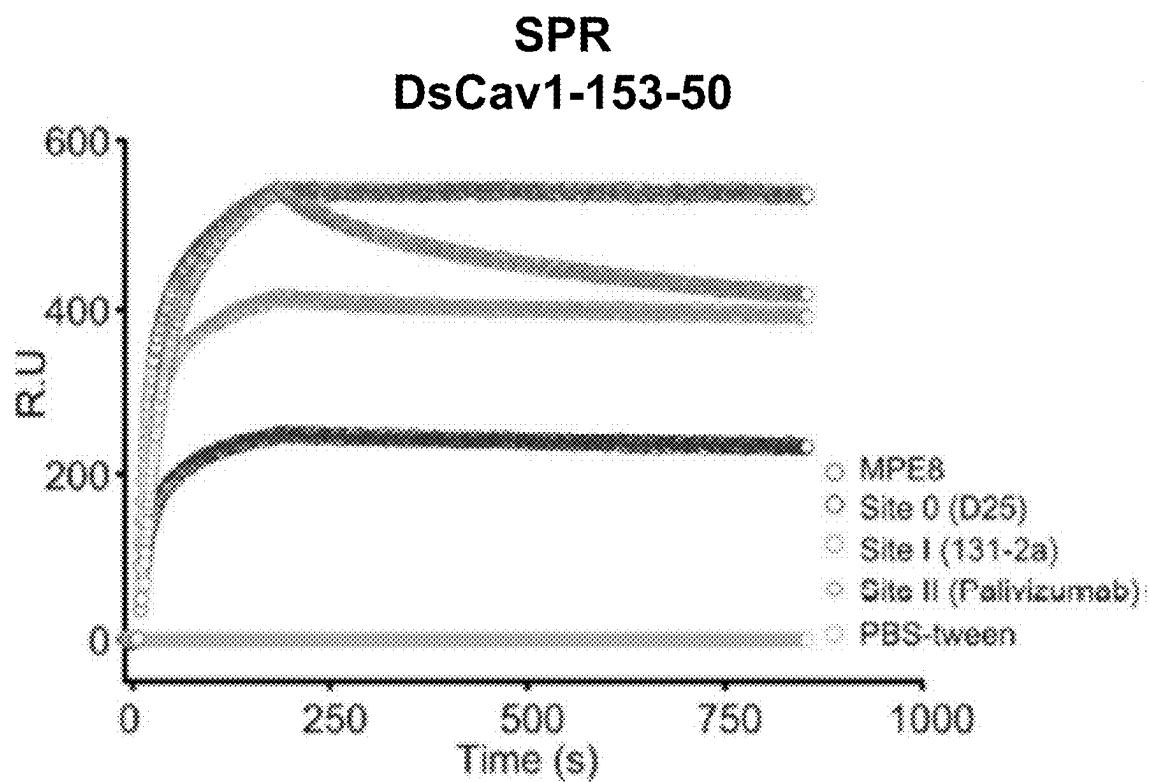


FIG. 6B

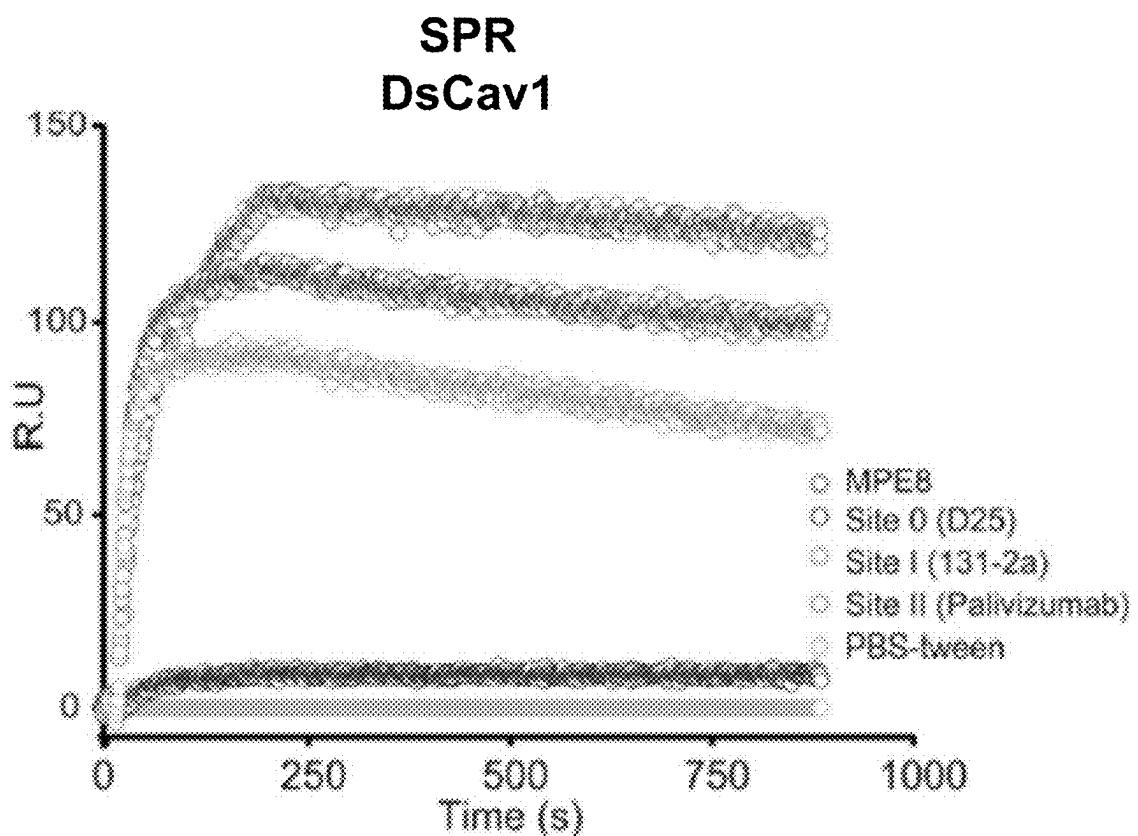


FIG. 6C

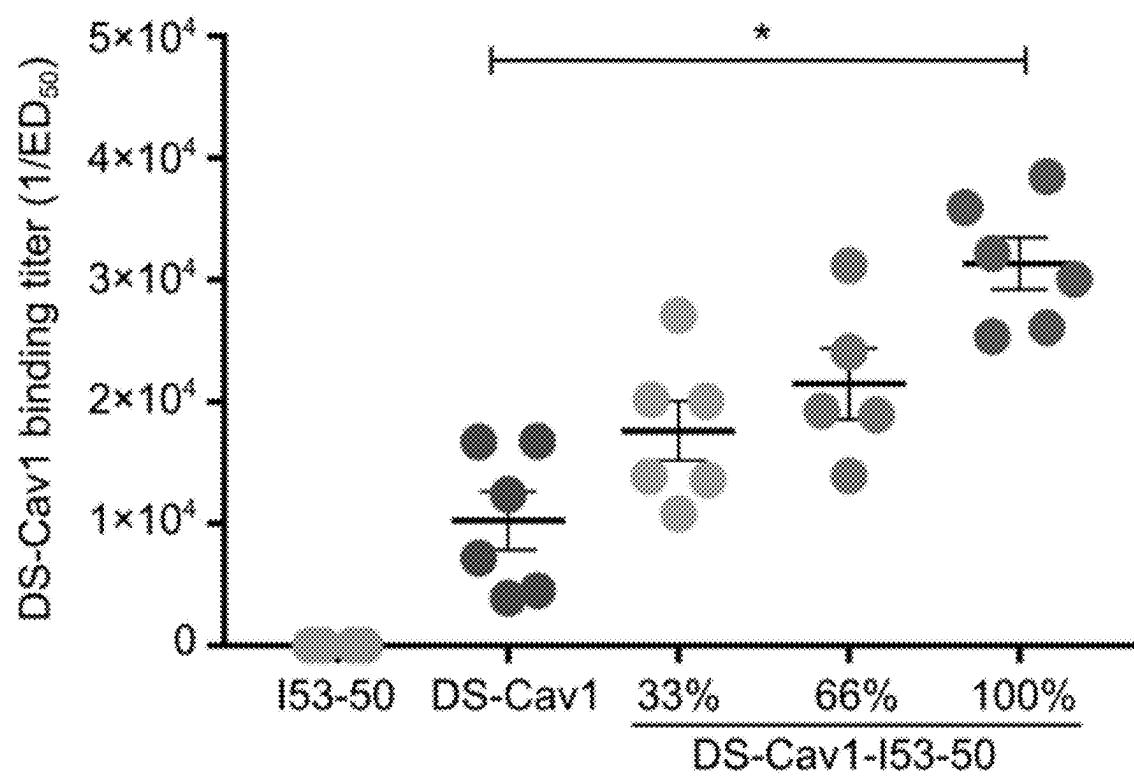


FIG. 7

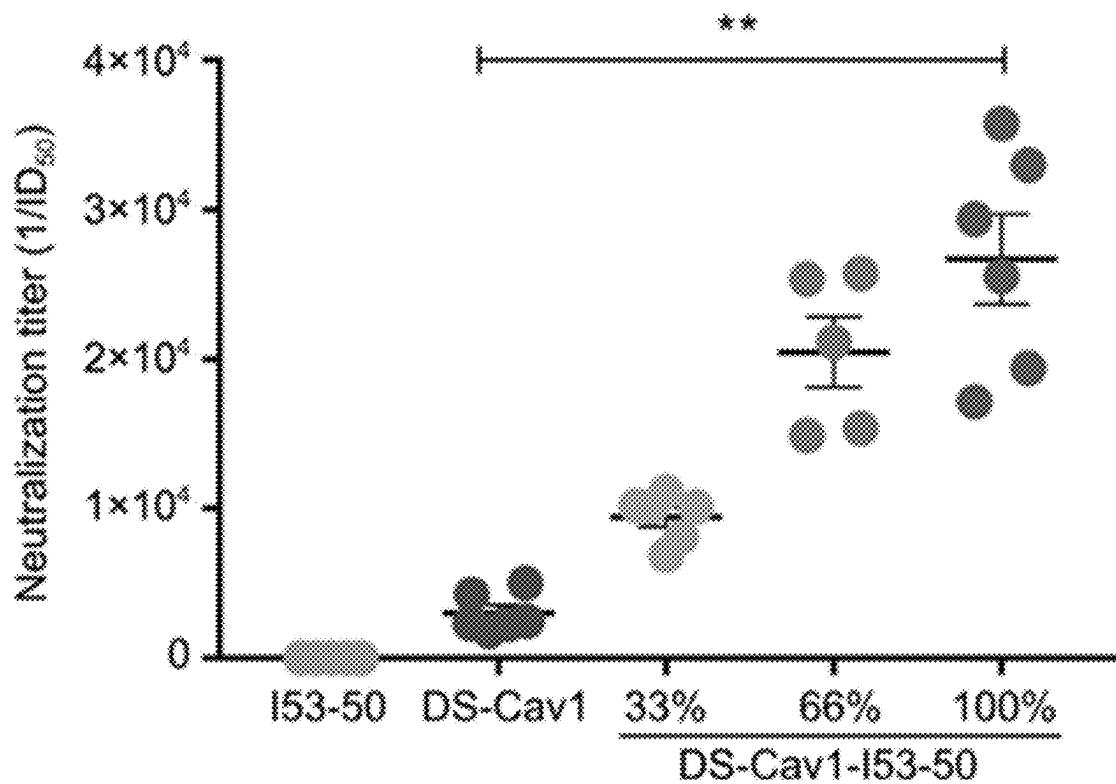


FIG. 8

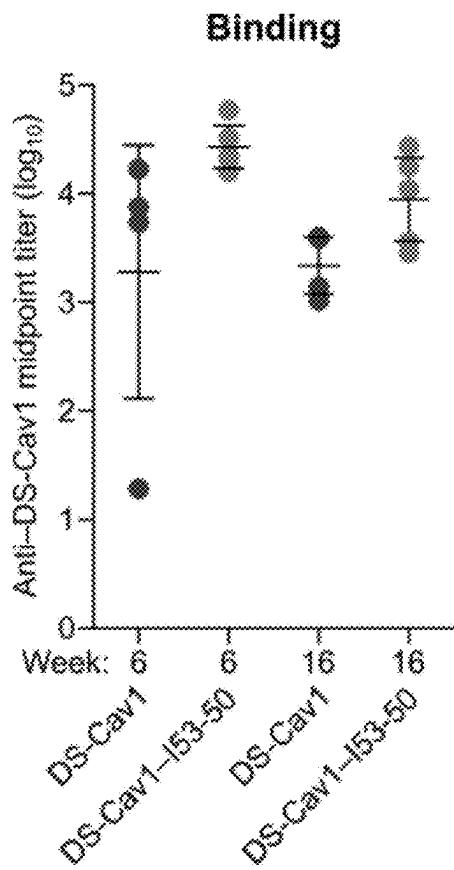


FIG. 9A

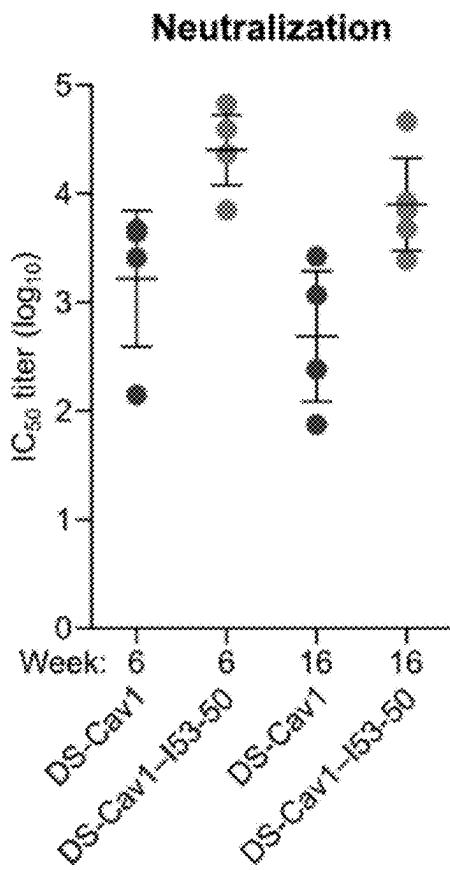


FIG. 9B

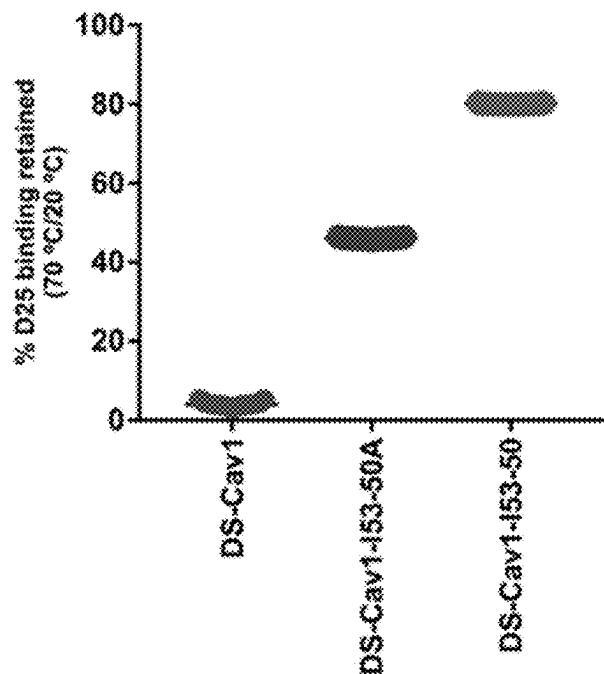


FIG. 10

DS-Cav1
FIG. 11A

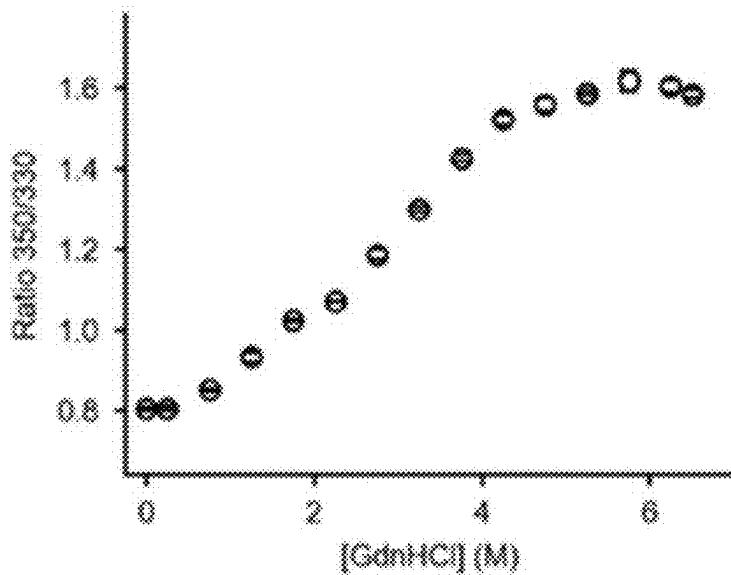


FIG. 11B

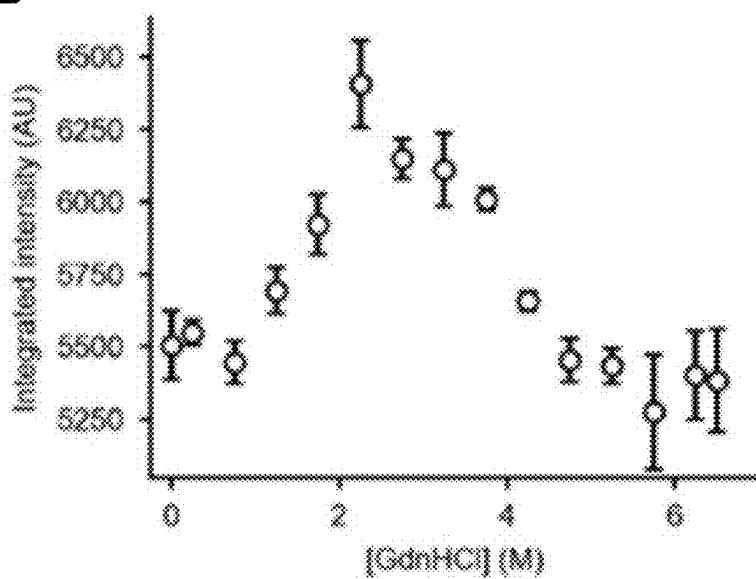


FIG. 11C

DS-Cav1-i53-50A

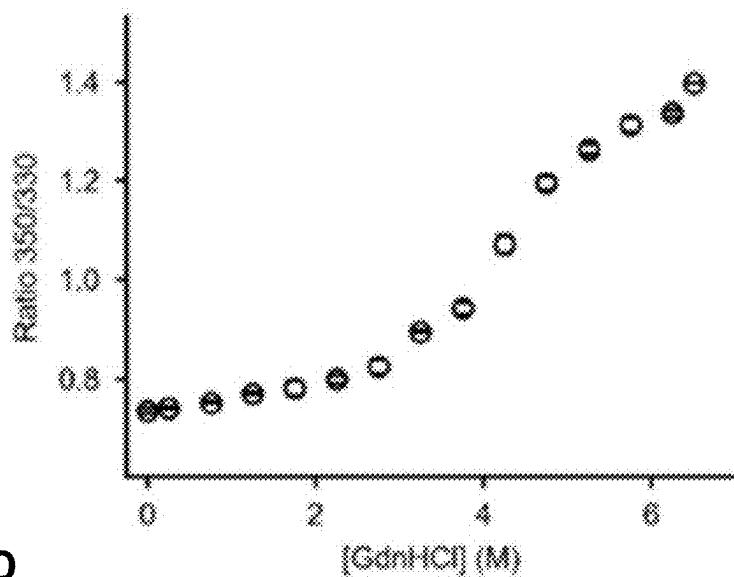


FIG. 11D

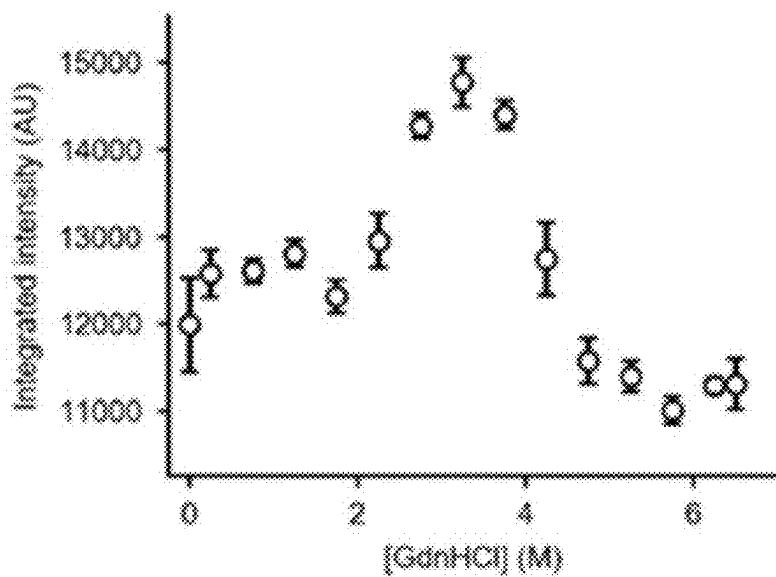


FIG. 11E

DS-Cav1-l53-50

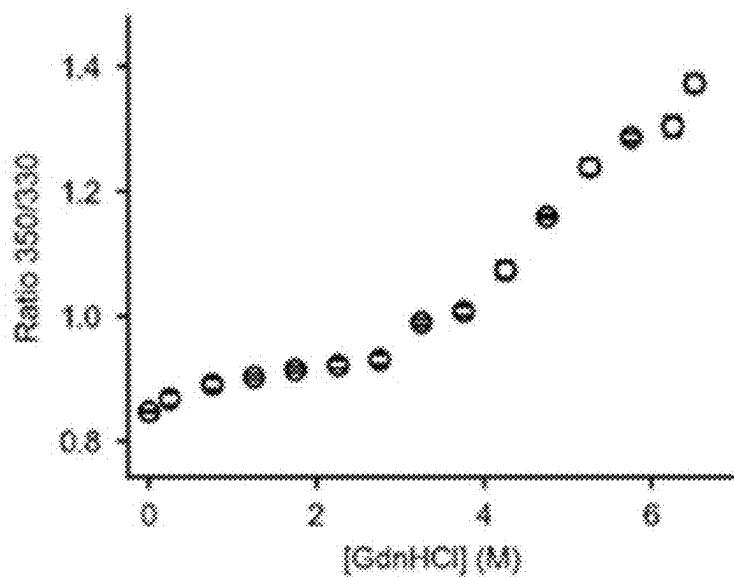


FIG. 11F

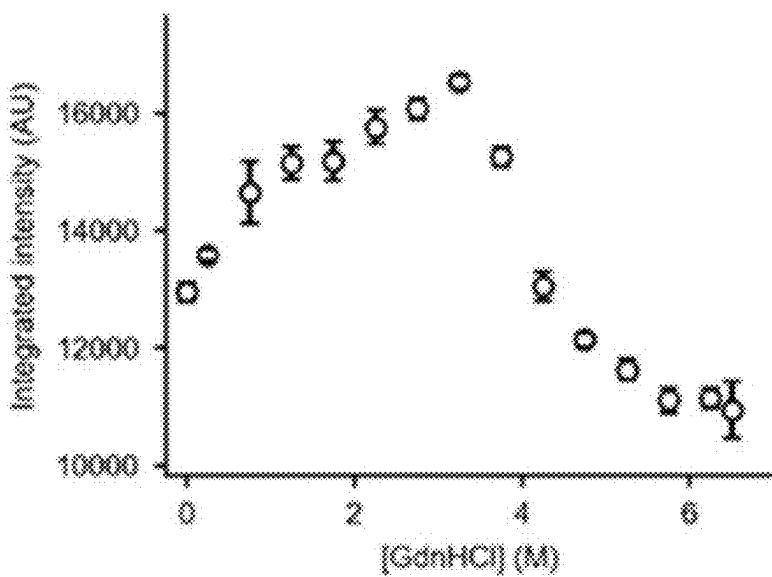


FIG. 11G

I53-50

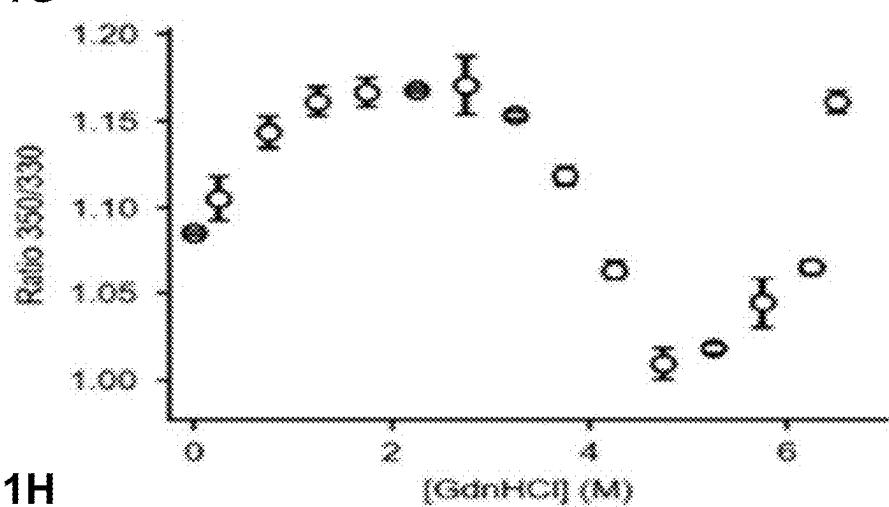


FIG. 11H

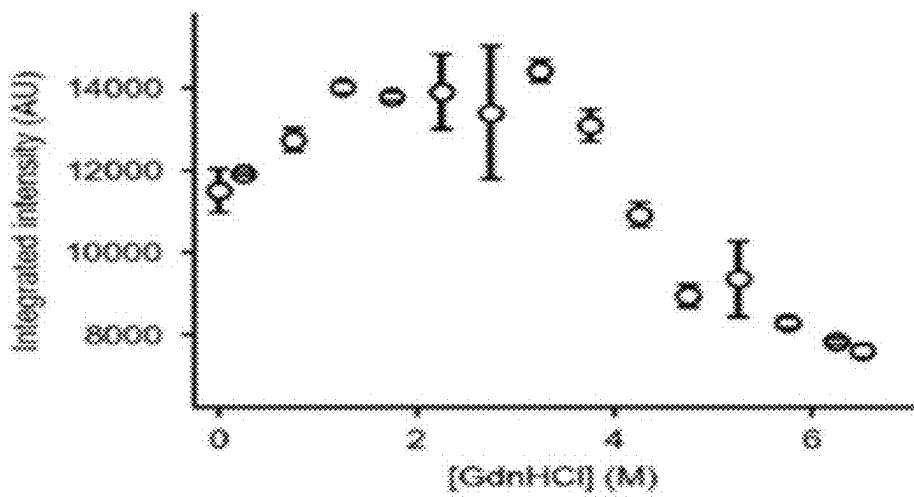


FIG. 11I

I53-50A

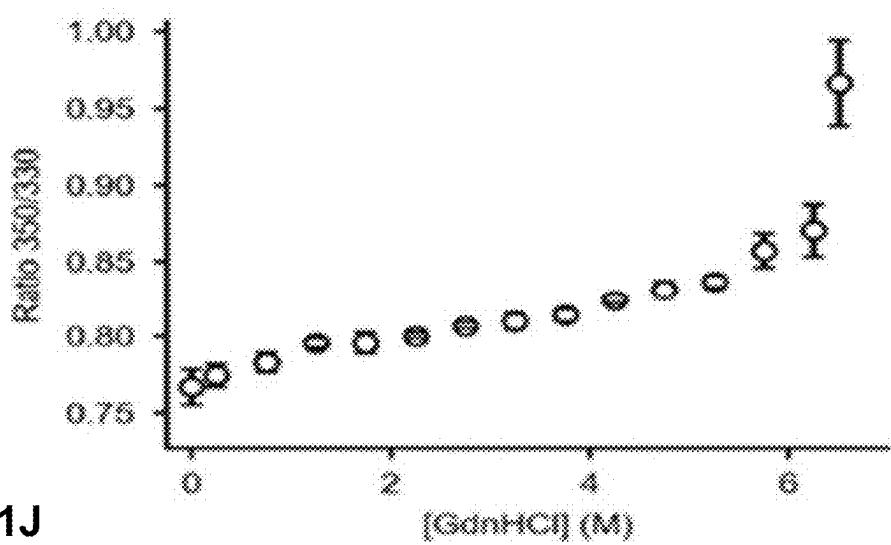
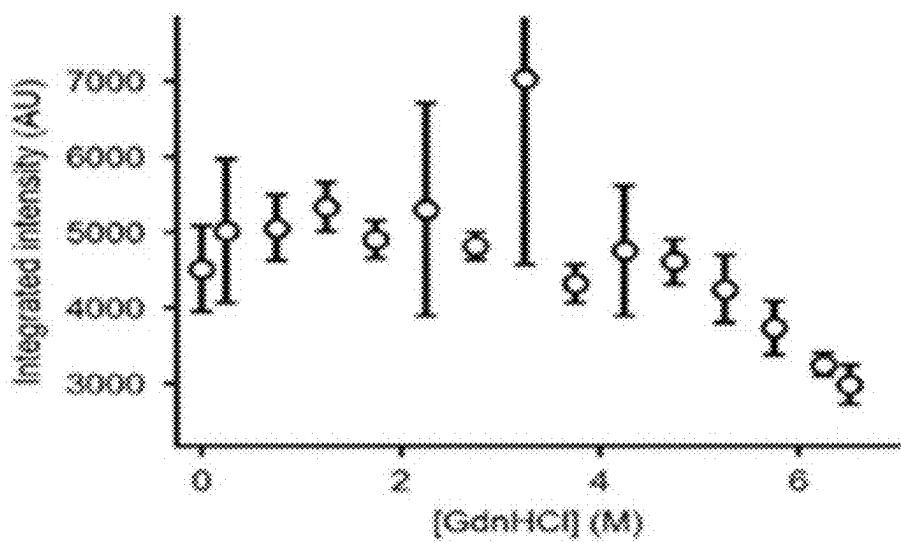


FIG. 11J



**SELF-ASSEMBLING PROTEIN
NANOSTRUCTURES DISPLAYING
PARAMYXOVIRUS AND/OR PNEUMOVIRUS
F PROTEINS AND THEIR USE**

BACKGROUND

[0001] 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.

**REFERENCE TO SEQUENCE LISTING
SUBMITTED ELECTRONICALLY**

[0002] The instant application contains a Sequence Listing which has been submitted electronically and is hereby incorporated by reference in its entirety. The Sequence Listing is contained in the file created on Jun. 27, 2023, having the file name "17-341-PCT.xml" and is 222,365 bytes in size.

SUMMARY OF THE INVENTION

[0003] In one aspect, nanostructures are provided comprising:

- [0004]** (a) a plurality of first assemblies, each first assembly comprising a plurality of identical first polypeptides;
- [0005]** (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;
- [0006]** wherein the plurality of first assemblies non-covalently interact with the plurality of second assemblies to form a nanostructure; and
- [0007]** 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.
- [0008]** In one embodiment, (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 100% identity along its full length to the amino acid sequence of a polypeptide selected from the group consisting of SEQ ID NOS: 1-51; and
- [0009]** (b) the second polypeptides comprise a polypeptide having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of a polypeptide selected from the group consisting of SEQ ID NOS: 1-51.
- [0010]** In another embodiment, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, comprise a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of a polypeptide selected from the group consisting of SEQ ID NOS: 53, 61-68, and 101.
- [0011]** In various embodiments:
- [0012]** (a) the first polypeptide comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of T33-31A (SEQ ID NO:51) and the second polypeptide comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of T33-09B/T33-31B (SEQ ID NO:44);
- [0013]** (b) the first polypeptide comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of T33-15B (SEQ ID NO:46) and the second polypeptide comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of T33-15A (SEQ ID NO:45);
- [0014]** (c) the first polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of a polypeptide selected from the group consisting of I53-50A (SEQ ID NO:7), I53-50A.1 (SEQ ID NO:29), I53-50A. INegT2 (SEQ ID NO:30), and I53-50A.1PosT1 (SEQ ID NO:31), and the second polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of a polypeptide selected from the group consisting of I53-50B (SEQ ID NO:8), I53-50B.1 (SEQ ID NO:32), I53-50B. INegT2 (SEQ ID NO:33), and I53-50B.4PosT1 (SEQ ID NO:34); or
- [0015]** (d) the first polypeptide comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of I32-28A (SEQ ID NO:21) and the second polypeptide comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of I32-28B (SEQ ID NO:22).
- [0016]** In one embodiment, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, are expressed as a fusion protein with the first polypeptides. In one such embodiment, the plurality of first assemblies each comprise identical first polypeptides; in another such embodiment, the plurality of first assemblies in total comprise two or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof. In another embodiment, only a subset of the first polypeptides comprise a fusion protein with an F protein or antigenic fragment thereof. In a further embodiment, each first assembly comprises a homotrimer of the first polypeptide.
- [0017]** In another embodiment, the fusion protein comprises an amino acid linker positioned between the first polypeptide and the paramyxovirus and/or pneumovirus F proteins, or antigenic fragment thereof. In one such embodiment, the fusion protein comprises an amino acid linker positioned between the first polypeptide and the paramyxovirus F proteins, or antigenic fragment thereof.

In one embodiment the amino acid linker sequence comprises one or more trimerization domain; in another embodiment the amino acid linker sequence comprises a Gly-Ser linker.

[0018] In various embodiments, the first polypeptides comprise or consist of first 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 a polypeptide selected from the group consisting of DS-Cav1-foldon-T33-31A (SEQ ID NO: 69), DS-Cav1-T33-31A (SEQ ID NO:70), DS-Cav1-foldon-T33-15B (SEQ ID NO: 71), DS-Cav1-T33-15B (SEQ ID NO:72), DS-Cav1-foldon-I53-50A (SEQ ID NO: 73), DS-Cav1-I53-50A (SEQ ID NO:74), and DS-Cav1-I32-28A (SEQ ID NO:75). In other embodiments,

[0019] (a) when each first polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of DS-Cav1-foldon-T33-31A (SEQ ID NO:69) or DS-Cav1-T33-31A (SEQ ID NO:70), each second polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of T33-31B (SEQ ID NO:44);

[0020] (b) when each first polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of DS-Cav1-foldon-T33-15B (SEQ ID NO:71) or DS-Cav1-T33-15B (SEQ ID NO:72), each second polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of T33-15A (SEQ ID NO:45);

[0021] (c) when each first polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of DS-Cav1-foldon-I53-50A (SEQ ID NO:73) or DS-Cav1-I53-50A (SEQ ID NO:74), each second polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of a polypeptide selected from the group consisting of I53-50B (SEQ ID NO: 8), I53-50B.1 (SEQ ID NO:32), I53-50B. 1 NegT2 (SEQ ID NO:33), or I53-50B.4PosT1 (SEQ ID NO:34); or

[0022] (d) when each first polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of DS-Cav1-I32-28A (SEQ ID NO:75), each second polypeptide has at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of I32-28B.

[0023] In one embodiment, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of DS-Cav1 (SEQ ID NO:53). In one such embodiment, each first polypeptide comprises a fusion polypeptide of a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or

100% identity along its full length to the amino acid sequence of DS-Cav1 linked via an amino acid linker to a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of SEQ ID NO:7 (I53-50A). In another embodiment, the amino acid linker comprises a Gly-Ser linker. In a further embodiment, each fusion protein comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence selected from the group consisting of SEQ ID NOS: 69-100. In a specific embodiment, each fusion protein comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of SEQ ID NO:76 (F10). In other embodiments, each second polypeptide comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence selected from the group consisting of I53-50B (SEQ ID NO: 8), I53-50B.1 (SEQ ID NO:32), I53-50B. 1 NegT2 (SEQ ID NO:33), or I53-50B.4PosT1 (SEQ ID NO:34). In a specific embodiment, each second polypeptide comprises a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of I53-50B.4PosT1 (SEQ ID NO:34).

[0024] In other aspects, recombinant expression nucleic acids expressing the first polypeptide fusions, recombinant expression vectors comprising the recombinant nucleic acids linked to a promoter, and recombinant host cells comprising the recombinant expression vectors are provided.

[0025] Also provided are immunogenic compositions comprising the nanostructure of any embodiment or combination of embodiments disclosed herein, and a pharmaceutically acceptable carrier. In one embodiment, the immunogenic compositions may further comprise an adjuvant.

[0026] In other aspects, methods for generating an immune response to RSV F protein in a subject, or for treating or limiting a RSV infection in a subject are provided, comprising administering to the subject in need thereof an effective amount of the nanostructure or immunogenic composition of embodiment or combination of embodiments disclosed herein to generate the immune response or to treat or prevent RSV infection in the subject.

[0027] Also provided are processes assembling the nanostructures of any embodiment or combination of embodiments disclosed herein, comprising mixing two or more nanostructures components in aqueous conditions to drive spontaneous assembly of the desired nanostructures.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings.

[0029] FIG. 1 shows a schematic diagram of the production of antigen-bearing nanostructures by *in vitro* assembly. The two components or building blocks of a given nanostructure can be expressed and purified individually, which allows assembly of the nanostructure to be initiated by

mixing the purified components in vitro, a process referred to as in vitro assembly. In some embodiments, the two components of the nanostructure may be expressed in different expression hosts (e.g., human HEK293F cells or bacterial *E. coli* cells). The figure schematically depicts assembly of a 120-subunit nanostructure bearing 20 trimeric antigens (60 antigen subunits) via in vitro assembly of an antigen-nanostructure trimer fusion protein produced in HEK293F cells and a nanostructure pentamer protein produced in *E. coli*.

[0030] FIG. 2 shows graphs illustrating detection of secreted DS-Cav1, DS-Cav1-foldon-T33-31A, and DS-Cav1-T33-31A fusion proteins in tissue culture supernatants. ELISA assays were performed on tissue culture supernatants from cells expressing DS-Cav1 (top), DS-Cav1-foldon-T33-31A/T33-31B (bottom left), and DS-Cav1-T33-31A/T33-31B (bottom right). Four different monoclonal antibodies that bind RSV F were used to evaluate the presence of DS-Cav1 or DS-Cav1 fusion proteins in the supernatants. The results confirm the secretion of proteins comprising well-folded RSV F antigen.

[0031] FIG. 3 shows size-exclusion chromatography of DS-Cav1-I53-50A. Protein purified from tissue culture supernatants by immobilized metal affinity chromatography was applied to a SuperoseTM 6 10/300 GL size exclusion column. The protein eluted as a single, monodisperse species.

[0032] FIG. 4 shows size exclusion chromatography of in vitro-assembled DS-Cav1-I53-50 nanostructures. Purified DS-Cav1-I53-50A and I53-50B.4PT1 proteins were mixed at an approximately 1:1 molar ratio, incubated overnight at 4° C., and then applied to a Sephadex 5-500 16/60 HR size exclusion column. The assembled nanostructure eluted as a single, monodisperse peak around 65 mL, while excess DS-Cav1-I53-50A trimeric component eluted around 90 mL.

[0033] FIG. 5 shows a negative stain electron micrograph and two-dimensional class averages of in vitro-assembled DS-Cav1-I53-50 nanostructures. In vitro-assembled DS-Cav1-I53-50 nanostructures, purified by size exclusion chromatography, were imaged by negative stain electron microscopy (top). Averaging many nanostructures yielded two-dimensional class averages (bottom) that indicate that the I53-50 portion of the nanostructures is highly ordered and consistent, while the precise three-dimensional position of the displayed antigen varies slightly due to the flexible nature of the linker between the DS-Cav1 and I53-50A domains of the DS-Cav1-I53-50A fusion protein.

[0034] FIG. 6A-6C show a series of graphs depicting the antigenicity of DS-Cav1-I53-50 nanostructures. Analysis of purified DS-Cav1-I53-50 nanostructures by ELISA (FIG. 6A) using four RSV F-specific monoclonal antibodies, including the prefusion-specific antibodies MPE8, D25, and RSD5, indicated that the DS-Cav1 antigen is correctly folded and maintained in the prefusion state when multivalently displayed on DS-Cav1-I53-50 nanostructures. This finding was confirmed by surface plasmon resonance measurements using multiple RSV F-specific antibodies (FIG. 6B-6C), which, when compared to trimeric DS-Cav1, further suggested that multivalent display of DS-Cav1 results in an avidity effect that reduces the dissociation rate of the antibodies.

[0035] FIG. 7 is a graph depicting DS-Cav1-specific serum antibody titers from mice immunized with DS-Cav1-

I53-50 nanostructures. Groups of mice were immunized with I53-50 nanostructures lacking additional antigen, trimeric DS-Cav1, or I53-50 nanostructures bearing DS-Cav1 antigen at 33%, 66%, or 100% valency. DS-Cav1-specific serum antibody titers were measured by ELISA on plates coated with DS-Cav1. Serum antibody titers for each mouse are plotted as circles, with the geometric mean within each group plotted as a horizontal line.

[0036] FIG. 8 is a graph depicting serum neutralization activity elicited by immunization with DS-Cav1-I53-50 nanostructures. Groups of mice were immunized with I53-50 nanostructures lacking additional antigen, trimeric DS-Cav1, or I53-50 nanostructures bearing DS-Cav1 antigen at 33%, 66%, or 100% valency. Neutralization titers for each mouse are plotted as circles, with the geometric mean within each group plotted as a horizontal line.

[0037] FIG. 9A-9B are graphs depicting immunogenicity in a primate immune system elicited by immunization with DS-Cav1-foldon I53-50 nanostructures. Rhesus macaques were injected at weeks 0 and 4 with either free DS-Cav1 trimer or DS-Cav1-foldon-I53-50 nanostructures displaying DS-Cav1 at 100% valency. In both cases, the dose of DS-Cav1 antigen was 50 µg, and the immunogens were formulated with the MF59-like, squalene-based oil-in-water emulsion adjuvant SWE. Sera obtained from the animals at weeks 6 and 16 were evaluated for anti-DS-Cav1 antibody titers (FIG. 9A) and RSV-neutralizing antibody titers (FIG. 9B).

[0038] FIG. 10 is a graph depicting the physical stability of DS-Cav1 when fused to I53-50A and/or when further assembled into the icosahedral nanostructure. Samples of trimeric DS-Cav1, trimeric DS-Cav1-foldon-I53-50A, and DS-Cav1-foldon-I53-50 nanostructures containing equivalent concentrations of DS-Cav1 were split into four aliquots and incubated at 20, 50, 70 or 80° C. for 1 hour. After cooling to room temperature, D25 binding was assayed by surface plasmon resonance (SPR).

[0039] FIG. 11A-11J are graphs depicting physical stability of the nanostructures. Chemical denaturation in guanidine hydrochloride (GdnHCl), monitored by intrinsic tryptophan fluorescence, was used as a second, antibody-independent technique to evaluate physical stability of trimeric DS-Cav1 (FIG. 11A-11B), DS-Cav1-foldon-I53-50A (FIG. 11C-11D), DS-Cav1-foldon-I53-50 (FIG. 11E-11F), I53-50 (FIGS. 11G-11H), and I53-50A (FIG. 11I-11J). The data indicate superior physical stability of the DS-Cav1 antigen when genetically fused to the I53-50A nanostructure component.

DETAILED DESCRIPTION OF THE INVENTION

[0040] All references cited are herein incorporated by reference in their entirety. Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991, Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutscher, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990, Academic Press, San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Ed. (R.I. Freshney.

1987. Liss, Inc. New York, NY), *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E. J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX).

[0041] As used herein, the singular forms “a”, “an” and “the” include plural referents unless the context clearly dictates otherwise. “And” as used herein is interchangeably used with “or” unless expressly stated otherwise.

[0042] As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

[0043] As used herein, “about” means+/-5% of the recited parameter.

[0044] All embodiments of any aspect of the invention can be used in combination, unless the context clearly dictates otherwise.

[0045] Unless the context clearly requires otherwise, throughout the description and the claims, the words ‘comprise’, ‘comprising’, and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”. Words using the singular or plural number also include the plural and singular number, respectively. Additionally, the words “herein,” “above,” and “below” and words of similar import, when used in this application, shall refer to this application as a whole and not to any particular portions of the application.

[0046] The description of embodiments of the disclosure is not intended to be exhaustive or to limit the disclosure to the precise form disclosed. While the specific embodiments of, and examples for, the disclosure are described herein for illustrative purposes, various equivalent modifications are possible within the scope of the disclosure, as those skilled in the relevant art will recognize.

[0047] In a first aspect, the disclosure provides nanostructures, comprising:

[0048] (a) a plurality of first assemblies, each first assembly comprising a plurality of identical first polypeptides;

[0049] (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;

[0050] wherein the plurality of first assemblies non-covalently interact with the plurality of second assemblies to form a nanostructure; and

[0051] 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.

[0052] Self-assembling polypeptide nanostructures are disclosed herein that multivalently display paramyxovirus and/or pneumovirus F proteins on the nanostructure exteriors. Multiple copies of pairs of first and second polypeptides are able to self-assemble to form nanostructures, such as icosahedral nanostructures. The nanostructures include symmetrically repeated, non-natural, non-covalent polypeptide-

polypeptide interfaces that orient a first assembly and a second assembly into a nanostructure, such as one with an icosahedral symmetry.

[0053] The nanostructures of the invention are synthetic, in that they are not naturally occurring. The first polypeptides and the second polypeptides are non-naturally occurring proteins that can be produced by any suitable means, including recombinant production or chemical synthesis. Each member of the plurality of first polypeptides is identical to each other (though when the first polypeptide is present as a fusion polypeptide with one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, the F protein or antigenic fragment thereof may differ from one first polypeptide to another), and each member of the plurality of second polypeptides is identical to each other. The first proteins and the second proteins are different. There are no specific primary amino acid sequence requirements for the first and second polypeptides. US published patent application 20160122392 and published PCT application WO2014/124301 describe methods for designing synthetic nanostructures, where the nanostructures are not dependent on specific primary amino acid sequences of the first and second polypeptides.

[0054] A plurality (2, 3, 4, 5, 6, or more) of first polypeptides self-assemble to form a first assembly, and a plurality (2, 3, 4, 5, 6, or more) of second polypeptides self-assemble to form a second assembly. A plurality of these first and second assemblies then self-assemble non-covalently via the designed interfaces to produce the nanostructures.

[0055] The number of first polypeptides in the first assemblies may be the same or different than the number of second polypeptides in the second assemblies. In one exemplary embodiment, the first assembly comprises trimers of the first polypeptides, and the second assembly comprises dimers of the second polypeptides. In a further exemplary embodiment, the first assembly comprises trimers of the first polypeptides, and the second assembly comprises trimers of the second polypeptides. In a further exemplary embodiment, the first assembly comprises trimers of the first polypeptides, and the second assembly comprises pentamers of the second polypeptides.

[0056] The first and second polypeptides may be of any suitable length for a given purpose of the resulting nanostructure. In one embodiment, the first polypeptides and the second polypeptides are typically between 30-250 amino acids in length; the length of the first polypeptides and the second polypeptides may be the same or different. In various further embodiments, the first polypeptides and the second polypeptides are between 30-225, 30-200, 30-175, 50-250, 50-225, 50-200, 50-175, 75-250, 75-225, 75-200, 75-175, 100-250, 100-225, 100-200, 100-175, 125-250, 125-225, 125-200, 125-175, 150-250, 150-225, 150-200, and 150-175 amino acids in length.

[0057] The isolated polypeptides of SEQ ID NOS: 1-51 were designed for their ability to self-assemble in pairs to form nanostructures, such as icosahedral nanostructures. The design involved design of suitable interface residues for each member of the polypeptide pair that can be assembled to form the nanostructure. The nanostructures so formed include symmetrically repeated, non-natural, non-covalent polypeptide-polypeptide interfaces that orient a first assembly and a second assembly into a nanostructure, such as one with an icosahedral symmetry. Thus, in one embodiment the first and second polypeptides are selected from the group SEQ ID NOS: 1-51. In each case, the N-terminal methionine residue is optional.

TABLE 1

Name	Amino Acid Sequence	Identified interface residues
I53-34A SEQ ID NO: 1	(M) EGMDPLAVLAESRLLPPLLTVRGGEDLAGLATVLELMGV GALEITLRLTEKGLEALKALKSGLLLGGAGTVRSPKEAEAAL EAGAAFLVSPGLLEEVAAALAQARGVPYLPGVLTPTEVERAL ALGLSALKFFPAEPFQGVRVLRAYAEVFPEVRFPLPTGGIKE EHLPHYAALPNLLAVGGSWLQGDLAAVMKKVAAKALLSP QAPG	I53-34A: 28, 32, 36, 37, 186, 188, 191, 192, 195
I53-34B SEQ ID NO: 2	(M) TKKVGIVDTTFARVDMAAIRTLKALSPNIKIIRKTV PGIKDLPVACKLLEEEGGDIVMALGMPGKAEKDKVCAHEA SLGLMLAQMLMTNKHIIIEFVHEDEAKDDDELILALVRAIE HAANVYYLLFKPEYLTRMAGKGLRQGFEDAGPARE	I53-34B: 19, 20, 23, 24, 27, 109, 113, 116, 117, 120, 124, 148
I53-40A SEQ ID NO: 3	(M) TKKVGIVDTTFARVDMASAAITLKMESPNIKIIRKTV PGIKDLPVACKLLEEEGGDIVMALGMPGKAEKDKVCAHEA SLGLMLAQMLMTNKHIIIEFVHEDEAKDAELKILAARRAIE HALNVYYLLFKPEYLTRMAGKGLRQGFEDAGPARE	I53-40A: 20, 23, 24, 27, 28, 109, 112, 113, 116, 120, 124
I53-40B SEQ ID NO: 4	(M) STINNQLKALKVIPVIAIDNAEDIIPLGKVLAEGLPA AEITFRSSAAVKAMLLRSQAPEMLIGACTIILNGVQALAAK EAGATFVSPGFNPNTVRACQIIGIDIVPGVNNPSTVEAAL EMGLTTLKFFPAEASGGISMVKSLVGPYCDIRLMPTGGITP SNIDNYLAIPQVLACGGTWMVDKLVTNGEWEDEIARLTREI VEQVNP	I53-40B: 47, 51, 54, 58, 74, 102
I53-47A SEQ ID NO: 5	(M) PIFTLNTNIKATDVPSDFLSLTSRLVGLILSKPGSYVA VHINTDQQLSFGGSTNPAAGTLMISIGGIEPSKNRDHSAVL FDHLNAMLGIPKPNRMYIHVNLNQDDVGWNGTTF	I53-47A: 22, 25, 29, 72, 79, 86, 87
I53-47B SEQ ID NO: 6	(M) NQHSHKDYEVTRIAIVVRARWHADIVDACVEAFIAMA IGGDRFAVDVFDPVPGAYEIPHLHARTLAETGRYGAVLGTA VNGGIYRHEPVASAVIDGMNNVQLSTGVPVLSAVLTPHRY DSAEHHHRFAAHFAVKGVVEAACRACIEILAAREKIAA	I53-47B: 28, 31, 35, 36, 39, 131, 132, 135, 139, 146
I53-50A SEQ ID NO: 7	(M) KMEELFKKKHIVAVLRLANSVEEAIEKAVAVFAGGVHLI EITFTVDPADTVIKALSVLKKEKGAIIGAGTVTSVEQCRKAV ESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAM KLGHТИLKFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNL NVCEWFKAGVLAvgvgsalvkgtpdevrekafvekirgc TE	I53-50A: 25, 29, 33, 54, 57
I53-50B SEQ ID NO: 8	(M) NQHSHKDYEVTRIAIVVRARWHAIEVDACVSafeamad IGGDRFAVDVFDPVPGAYEIPHLHARTLAETGRYGAVLGTA VNGGIYRHEPVASAVIDGMNNVQLSTGVPVLSAVLTPHRY DSDAHTLLFLALFAVKGMEEAACRACVEILAAREKIAA	I53-50B: 24, 28, 36, 124, 125, 127, 128, 129, 131, 132, 133, 135, 139
I53-51A SEQ ID NO: 9	(M) FTKSGDDGNTNVINKRVRGKDPLVNPLGDLDELNFIG FAISKIPWEDMKKDLERVQVLFIEIGEDLSTQSSKKKIDES YVLWLLAATAIYRIESGPVKLFVIPGGSEEASVLHVTRSA RRVERNAVYKTKELPEINRMIIVYLNRLSSLFAMALVANK RRNQSEKUYEIGKSW	I53-51A: 80, 83, 86, 87, 88, 90, 91, 94, 166, 172, 176
I53-51B SEQ ID NO: 10	(M) NQHSHKDYEVTRIAIVVRARWHAIEVDQCVRafeamad AGGDRFAVDVFDPVPGAYEIPHLHARTLAETGRYGAVLGTA VNGGIYRHEPVASAVIDGMNNVQLSTGVPVLSAVLTPHRY SSREHHFEFREHFMVKGVEAAAACITILAAREKIAA	I53-51B: 31, 35, 36, 40, 122, 124, 128, 131, 135, 139, 143, 146, 147
I52-03A SEQ ID NO: 11	(M) GHTKGPTPQQHDGSALRIGIVHARWNKTIIMPLLIGTI AKLLECGVKASNIVVQSVPGSWELPIAVQRLYSASQLQTPS SGPSLSAGDLGGSSTTDLALPTTASSTGPFDALIAIGVL IKGETMHFYIADS VSHGLMRVQLDTGVPVIFGVLT QAKARAGVIEGSHNHGEDWGLAAVEMGVRRDWAAAGKTE	I52-03A: 28, 32, 36, 39, 44, 49
I52-03B SEQ ID NO: 12	(M) YEVDHADYDYLGYRKDYAAEASDIADLVR SRTPEA SSLLDVACGTGTHLEHFTKEFGDTAGLELSEDMTHARKRL PDATLHQGDMDRFQGLRKFSAVVSMFSSVGYLKTVAELGAA VASFAEHLEPGGVVVVEPWPFETFADGVWSADVRRDGR VARVSHSVREGNATRMEVHTVADPGKGVVRHFSDVHLITLF HQREYEAAFMAGLRVEYLEGGPSGRGLFVGVP	I52-03B: 94, 115, 116, 206, 213

TABLE 1-continued

Name	Amino Acid Sequence	Identified interface residues
I52-32A SEQ ID NO: 13	(M) GMKEKFVLIITHGDFGKGLLSGAEVIIIGKQENVHTVGLNLGDNIEKVAKEVMRIIIAKLAEDKEIIIVVDLFGGSPFNIALEMMKTFDVKVITGINMPMLVELLTSINVYDTTELENISKIGKDGIKVIEKSSLKM	I52-32A: 47, 49, 53, 54, 57, 58, 61, 83, 87, 88
I52-32B SEQ ID NO: 14	(M) KYDGSKLRLIGILHARWNLEIIAALVAGAIKRLQEFGVKAENIIIETVPGSFELPYGSKLFVEKQKRLGPDAIPIGVLIKGSTMHFYEYICDSTTHQLMKLNFEGLGIPVIFGVLTCLTD EQAEARAGLIEGKMHNHGEDWGAAVEMATKFN	I52-32B: 19, 20, 23, 30, 40
I52-33A SEQ ID NO: 15	(M) AVKGLGEVDQKYDGSKLRLIGILHARWNRKIILALVAGAVLRLLEFGVKAENIIIETVPGSFELPYGSKLFVEKQKRLGPPLDAIPIGVLIKGSTMHFYEYICDSTTHQLMKLNFEGLGIPVIFGVLTCLTD EQAEARAGLIEGKMHNHGEDWGAAVEMATKFN	I52-33A: 33, 41, 44, 50
I52-33B SEQ ID NO: 16	(M) GANWYLDNESSRRLSFTSTKNADIAEVHRLFLVLHGKVDPKGLAEVEVETESTISITGIPLRDMLLRVLVPOVSKEPVQAQINAQLDMRPIINNLAPGAQLELRPLPTVSLRGKSHSYNAELLATR LDERRFQVVTLEPLVHIAQDFDMVRFAFNALRLVAGLSAVSL SVPVGAVLIFTAR	I52-33B: 61, 63, 66, 67, 72, 147, 148, 154, 155
I32-06A SEQ ID NO: 17	(M) TDYIRDGSAIKALSFAIIIAEADLRHIPQDLQRLAVRVIHACGMVDVANDLAFLSEGAAGKAGRALLAGAPILCDARMVAEGITRSRLPADNRVIYTLDPSVPPELAKIGNTRSAALDLWLPHIEGSIVAIIGNAPTALFRLFELLDAGAPKPALIIGMPVGFVGAAESKDELAANSRGVPYVIVRGRGGSAMTAANVALASERE	I32-06A: 9, 12, 13, 14, 20, 30, 33, 34
I32-06B SEQ ID NO: 18	(M) ITVFGLKSKLAPRREKLAEVIVYSSLHLGLDIPKGKHAI RFLCLEKEDYPYFPDRSDDYTVIEINLMAGRSEETKMLLIFLLFIALERLGIRAHDEVITIKEQPAHCWGRGRTGDSARDLDYDIYV	I32-06B: 24, 71, 73, 76, 77, 80, 81, 84, 85, 88, 114, 118
I32-19A SEQ ID NO: 19	(M) GSDLQKLQRFSTCDISDGLLNVYNIPGGYGPNLTAISPPQNSSSIVGTAYTVLFAPIDPRPAVNYIDSVPVNSILVLALEPHLQSQFHFPFIKITQAMYGGMSTRQYLKNSNGTUVFGRIRDVDEHRTLNHPVFAYGVGSCAPKAVVKAVGNTVQLKILTSDGVTQTCICPGDYTAGDNNGIVRIPVQETDISKLVTYIEKSIEVDRLVSEAIKNGLPAKAAQTARRMVLKDYI	I32-19A: 208, 213, 218, 222, 225, 226, 229, 233
I32-19B SEQ ID NO: 20	(M) SGMRVYLGADHAGYELKQAIIAFLKMTGHEPIDCGALRYDADDYPAFCIAAATRTVADPGSLGIVLGGSGNQEQIAANKVPGARCALAWSVQTAALAREHHNNAQLCIGGGRMHTLEEALRIVKAFVTPWSKAQRHQRRIDILAEYERTHEAPPVPGAPA	I32-19B: 20, 23, 24, 27, 117, 118, 122, 125
I32-28A SEQ ID NO: 21	(M) GDDARIAAIGDVDELNSQIGVLLAEPPLPDDVRAALSAIQHDLFDLGGELCIPGHAAITEDHLLRALWLWVHYNGQLPPL EEFILPGGARGAAALAHVCRTRVCCRERSIKALGASEPLNIA PAAYVNLLSDLLFVLARVLNRAAGGADVLWDRTRAH	I32-28A: 60, 61, 64, 67, 68, 71, 110, 120, 123, 124, 128
I32-28B SEQ ID NO: 22	(M) ILSAEQSFRTLHRPHGQAAALAFVREPAALAGVQRLRG LDSDGEQVWGEELLVRVPLLGEVDPFRSEIVRTPQGAELRP LTLTGERANAVAVSGATAAEGGEMAFAFQAFQAHLATPEAEG EGGAFAEVMVQAAAGVTLVVAMALPQGLAAGLPPA	I32-28B: 35, 36, 54, 122, 129, 137, 140, 141, 144, 148
I53-40A.1 SEQ ID NO: 23	(M) TKKVGIVDFTTFARVDMASAAITLKMESPNIKIIRKTV PGIKDLPVACKLLEEEGCDIVMALGMPGKKEKDVKCAHEA SLGLMLAQLMTNKHIIEFVPHDEAKDAAELKILAARRAIEHALNVYYLLFKPEYLTRMAGKGLRQGFEDAGPARE	I53-40A: 20, 23, 24, 27, 28, 109, 112, 113, 116, 120, 124
I53-40B.1 SEQ ID NO: 24	(M) DDINNOLKRLKVIPVIAIDNAEIIDPLGKVLAAENGLPA AEITFRSSAAVKAIMLLRSAQPEMLIGAGTILNGVQALAAK EAGADFVVS PGFNPTVRAQCIIIGIDIVPGVNNNSTVEQAL EMGLTTLKFFPAEASGGISMVKSLVGPYDIRLMPTGGITPDNIDNYLAIPQVLACGGTWMVDKKLVRNGEWDEIARLTREI VEQVNP	I53-40B: 47, 51, 54, 58, 74, 102
I53-47A.1 SEQ ID NO: 25	(M) PIFTLNTNIKADDVPSDFLSLTSRLVGLLILSKPGSYVA VHINTDQQLSFGGSTNPAAFGTLMISIGGIEPDKNRDHSAVL FDHLNAMLGIPKNRMYIHFVNLngDDVGWNGTTF	I53-47A: 22, 25, 29, 72, 79, 86, 87

TABLE 1-continued

Name	Amino Acid Sequence	Identified interface residues
I53-47A. 1NegT2 SEQ ID NO: 26	(M) PIFTLNTNIKADDVPSDFSLTSRLVGLILSEPGSYVA VHINTDQQLSFGGSTNPAAFGTLMSIGGIEPDKNEDHSAVL FDHLNAMLGIPKNRMYIHFVLDGDVGWNGTTF	I53-47A: 22, 25, 29, 72, 79, 86, 87
I53-47B. 1 SEQ ID NO: 27	(M) NQHSHKDHETVRIAVVRARWHADIVDACVEAFEIAMA IGGDRFAVDVFDVPGAYEIPHLHARTLAETGRYGAVLGTA VNGGIYRHEFVASAVIDGMMNVQLDTGVPVLSAVLTPHRYR DSDEHHRFFAAHFAVKGVVEARACIEILNAREKIAA	I53-47B: 28, 31, 35, 36, 39, 131, 132, 135, 139, 146
I53-47B. 1NegT2 SEQ ID NO: 28	(M) NQHSHKDHETVRIAVVRARWHADIVDACVEAFEIAMA IGGDRFAVDVEDVPGAYEIPHLHARTLAETGRYGAVLGTA VDGGIYDHEFVASAVIDGMMNVQLDTGVPVLSAVLTPHYE DSDEDHEFFAAHFAVKGVVEARACIEILNAREKIAA	I53-47B: 28, 31, 35, 36, 39, 131, 132, 135, 139, 146
I53-50A. 1 SEQ ID NO: 29	(M) KMEELFKKKHICAVLRLANSVEEAIEKAVAVFAGGVHLI EITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAV ESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAM KLGHDIKLKLPGEVVGPGQFVKAMKGPFPPNVKFVPTGGVLD NVCEWFKAQGLAVGVGDALVKGDPDEVREAKKFVKEKRC TE	I53-50A: 25, 29, 33, 54, 57
I53-50A. 1NegT2 SEQ ID NO: 30	(M) KMEELFKKKHICAVLRLANSVEEAIEKAVAVFAGGVHLI EITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAV ESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAM KLGHDIKLKLPGEVVGPGQFVKAMKGPFPPNVKFVPTGGVLD DVCEWFDAQGLAVGVGDALVKGDPDEVREAKKFVKEEIRGC TE	I53-50A: 25, 29, 33, 54, 57
I53-50A. 1Post1 SEQ ID NO: 31	(M) KMEELFKKKHICAVLRLANSVEEAIEKAVAVFAGGVHLI EITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAV ESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAM KLGHDIKLKLPGEVVGPGQFVKAMKGPFPPNVKFVPTGGVLD NVCKWFKAQGLAVGVGDALVKGDPDEVREAKKFVKKIRGC TE	I53-50A: 25, 29, 33, 54, 57
I53-50B. 1 SEQ ID NO: 32	(M) NQHSHKDHETVRIAVVRARWHAEIVDACVSAFEAMRD IGGDRFAVDVFDVPGAYEIPHLHARTLAETGRYGAVLGTA VNGGIYRHEFVASAVIDGMMNVQLDTGVPVLSAVLTPHRYR DSDAHTLLFLALFAVKGMEEARACVEILAAREKIAA	I53-50B: 24, 28, 36, 124, 125, 127, 128, 129, 131, 132, 133, 135, 139
I53-50B. 1NegT2 SEQ ID NO: 33	(M) NQHSHKDHETVRIAVVRARWHAEIVDACVSAFEAMRD IGGDRFAVDVEDVPGAYEIPHLHARTLAETGRYGAVLGTA VDGGIYDHEFVASAVIDGMMNVQLDTGVPVLSAVLTPHYE DSDADTLLFLALFAVKGMEEARACVEILAAREKIAA	I53-50B: 24, 28, 36, 124, 125, 127, 128, 129, 131, 132, 133, 135, 139
I53-50B. 4Post1 SEQ ID NO: 34	(M) NQHSHKDHETVRIAVVRARWHAEIVDACVSAFEAMRD IGGDRFAVDVEDVPGAYEIPHLHARTLAETGRYGAVLGTA VNGGIYRHEFVASAVINGMMNVQLNTGVPVLSAVLTPHNYD KSKAHTLLFLALFAVKGMEEARACVEILAAREKIAA	I53-50B: 24, 28, 36, 124, 125, 127, 128, 129, 131, 132, 133, 135, 139
I53-40A genus (SEQ ID NO: 35) (M) TKKGIVDFTFARVDMASAAILTLKMESPNIKIIRKTVPGIKDLPVACKLLEEGCDIVMA LGMPGK (A/K) EKDVKCAHEASLGLMLAQLMTNKHIIEVFVHDEAKDDAELKILAARRAIEHAL NYYYLFPKPEYLTRMAGKGLRQGFEDAGPARE		
I53-40B genus (SEQ ID NO: 36) (M) (S/D) (T/D) INNQLK(A/R) LKVIPVIAIDNAEDIPLGKVL AENGLPAAEITFRSSAAVKAIM LVRSAQPEMLIGAGTILNGVQLAAKEAGA (T/D) FVVSPGENPNT TVRACQIIGIDIVPGVN NPS TVE (A/Q) ALEMGLTTLKFFPAEASGGISMVKSLVGPYGD DIRLMPTGGITP (S/D) NIDNYLAIP QVLACGGTWMVDKKLV (T/R) NGEWDEIARLTREIVEQVNP		
I53-47A genus (SEQ ID NO: 37) (M) PIFTLNTNIK(T/D) DVPSDFSLTSRLVGLILS (K/E) PG SYVAVHINTDQQLSFGGSTN PAAFGTLMSIGGIEP (S/D) KN (R/E) DHS AVLEDHLNAMLGIPKNRMYIHFV (N/D) L (N/D) G DDVGWNGTTF		
I53-47B genus (SEQ ID NO: 38) (M) NQHSHKD (Y/H) ETVRIAVVRARWHADIVDACVEAFEIAMA AIGGDRFAVDVEDVPGAYEIP LHARTLAETGRYGAVLGTA VV (N/D) GGIY (R/D) HEFVASAVIDGMMNVQL (S/D) TG VPVLS AVLTPH (R/E) Y (R/E) DS (A/D) E (H/D) H (R/E) FFAAHFAVKGV VEARACIEIL (A/N) ARE KIAA		

TABLE 1-continued

Name	Amino Acid Sequence	Identified interface residues
I53-50A genus (SEQ ID NO: 39)		
(M) KMEELFKKHKIVAVLRLANSVEEAIKEKAVAVFAGGVHLIEITFTV ррDADTVIKALSVLKEKGA IIGAGTGTTSV ррQCRKAVESGAЕFIVSPHLDEEISQFCKEKGVFYMPGVMPTTELVKAMKLGH		
(T/D) ILKLFPGEVVGP (Q/E) FV (K/E) AMKGPFPNVKFVPTGGV (N/D) LD (N/D) VC (E/K) WF (K/D) AGVLAVGVG (S/K/D) ALV (K/E) G (T/D/K) PDEVRE (K/D) AK (A/E/K) FV (E/K) (K/E) IRGCTE		
I53-50B genus (SEQ ID NO: 40)		
(M) NQHSHKD (Y/H) ETVRIAVVRARWHAЕIVDACVSAFEAAM (A/R) DIGGDRFAVDV ррDVPAGA YEIPLHARTLAETGRYGAVLGTAFVV (N/D) GGIY (R/D) HEFVASAVI (D/N) GMMNVQL (S/D/N) TGVPVLSAVLTPH (R/E/N) Y (R/D/E) (D/K) S (D/K) A (H/D) TLLFLALPAVKGME AARACVEILAAREKIAA		
T32-28A (SEQ ID NO: 41)		
(M) GEVPIGDPKELNGMEAIAAVYLQPIEMEPRGIDLAASLADIHLEADIHALKNNPNGFPEGFW PYLTIAYALANADTGAIKTGTLMPPVADDGPHYGANIAMEKDGGFGVGTYALTFLISNPEKQG FGRHVDEETGVGKWFEPFVVVTYFFKTGTPK		
T32-28B (SEQ ID NO: 42)		
(M) SQAIGILELTSIAKGMELGAMLKSANVDLLVSKTISPGKFLLMLGGDIGAIQQAIETGTSQ AGEMLVDSLVLANIHPSVLPASIGLNSVDKRQAVGIVETWSVAACISAADLAVKGSNVTLVRVHM AFGIGGGKCYMVAGDVLDVAAAATASLAAGAKGLLVYASIIPRPHAEWMRQMVEG		
T33-09A (SEQ ID NO: 43)		
(M) EEVVLITVPSALV ррKIAHALVEERLAACVNIVPGLTSIYRWQGSV ррSDHELLLVLKTTTHA FPKLKERVKALHPYTVPEIVALPIAEGNREYLDWLRENTG		
T33-09B (SEQ ID NO: 44)		
(M) VRGIRGAITVEEDTPAIIAATIELLLKMLEANGIQSYEEELAAVIFTV ррTEDLTSAPPAEAAR LGMHRVPLLSAREVPV ррPGSLPRV ррVRLALWNTDTPQDRVRHVYLNEAVRLRPDLESQAQ		
T33-15A (SEQ ID NO: 45)		
(M) SKAKIGIVTVDRASAGITADISGKAIILALNLYLTSEWEPIYQVIPDEQDVIETTLIKMAD EQDCCLIVTTGGTGPAKRDV ррFEATEAVCDRMPGPGELEMRAESLKEVPTAILSRQTAGLRGDSL IVNLPGDPASISDCLLAVFPAPICYCIDLMEGPYLECNEAMIKPFRPKAK		
T33-15B (SEQ ID NO: 46)		
(M) VRGIRGAITV ррNSTDPTSIIATILLKMLEANGIQSYEEELAAVIFTV ррTEDLTSAPPAEAAR QJGMHRVPLLSAREVPV ррPGSLPRV ррVRLALWNTDTPQDRVRHVYLNEAVRLRPDLESQAQ		
T33-21A (SEQ ID NO: 47)		
(M) RITTGVGDKGSTRLLFGGEVWKDSPPIIEANGTLDELTFSIGEAKHYDEEMKGILEEIQNDI YKIMGEIGSKGKIEGISEEVLKLILRYMEMVNLSFVLPGGTLESAKLDVCRTIARRALRK VLTVTREFGIGAEAAYLLALSDLLFLALARVIEIEKNKLKEVRS		
T33-21B (SEQ ID NO: 48)		
(M) PHLVIEATANLLETSPGELLEQANKALFASGQFGEADIKSRFV ррTLEAYRQGTAAVERAYLH ACLSILDGRDIATRTLGGASLCAVLAEAVAGGEEGVQSVЕVREMERLSYAKRVVARQR		
T33-28A (SEQ ID NO: 49)		
(M) ESVNTSFLSPSLVTIRDFTDNGQFAVLRIGHTGFPADKGIDILCLDKMIGVRAAQIFLGGDTE DGPKGPHIRCV DIDDKHTYNAMV ррV ррLIVGTGASEVERETAAEAKLALRVALQVDIADEHSC VTQFEMKLREELLSSDSFHРРDКDEYYKDFL		
T33-28B (SEQ ID NO: 50)		
(M) PVIQTFSPLDHHKRLLIAIYRIVTRV ррVLGKPEDLVMMTFHDSTPMFFGSTDРVACVRV EALGGYGPSEPEKVTISVTAITAVCGIVADRFV ррLYFSPLHCGWNGTNF		
T33-31A (SEQ ID NO: 51)		
(M) EEVVLITVPSALV ррKIAHALVEERLAACVNIVPGLTSTYREEGSV ррSDHELLLVLKTTDA FPKLKERVKALHPYEVPEIVALPIAEGNREYLDWLRENTG		

[0058] Table 1 provides the amino acid sequence of the first and second polypeptides; the right hand column in Table 1 identifies the residue numbers in each exemplary polypeptide that were identified as present at the interface of resulting assembled nanostructures (i.e.: “identified interface residues”). As can be seen, the number of interface residues for the exemplary polypeptides of SEQ ID NO: 1-34 range from 4-13. In various embodiments, the first and

second polypeptides comprise an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its length, and identical at least at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13 identified interface positions (depending on the number of interface residues for a given polypeptide), to the amino acid sequence of a polypeptide selected from the group consisting of SEQ ID NOS: 1-34. In other embodiments, the first

and second polypeptides comprise an amino acid sequence that is at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical over its length, and identical at least at 20%, 25%, 33%, 40%, 50%, 60%, 70%, 75%, 80%, 90%, or 100% of the identified interface positions, to the amino acid sequence of a polypeptide selected from the group consisting of SEQ ID NOS:1-51.

[0059] As is the case with proteins in general, the polypeptides are expected to tolerate some variation in the designed sequences without disrupting subsequent assembly into nanostructures; particularly when such variation comprises conservative amino acid substitutions. As used here, “conservative amino acid substitution” means that: hydrophobic amino acids (Ala, Cys, Gly, Pro, Met, See, Sme, Val, Ile, Leu) can only be substituted with other hydrophobic amino acids; hydrophobic amino acids with bulky side chains (Phe, Tyr, Trp) can only be substituted with other

hydrophobic amino acids with bulky side chains; amino acids with positively charged side chains (Arg, His, Lys) can only be substituted with other amino acids with positively charged side chains; amino acids with negatively charged side chains (Asp, Glu) can only be substituted with other amino acids with negatively charged side chains; and amino acids with polar uncharged side chains (Ser, Thr, Asn, Gln) can only be substituted with other amino acids with polar uncharged side chains.

[0060] Table 2 lists surface amino acid residue numbers for each exemplary polypeptide of the invention denoted by SEQ ID NOS: 1-34. Thus, in various embodiments, 1 or more (at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or more) of these surface residues may be modified in the polypeptides of the invention. Residues in parentheses are optional.

TABLE 2

Name	Amino Acid Sequence	Surface residues not near interface
I53-34A SEQ ID NO: 1	(M) EGMDPLAVLAESRLLPLLTVRGGEDLAGLATVLELMGV GALEITLRTEKGLEALKALRKSGLLLGGAGTVRSPKEAEAAAL EAGAAFLVSPGLLEEVAAALAQARGVPYLPGVLTPTEVERAL ALGLSALKFFPAEPFGQGVRVLRAYAEVFPEVRFLPTGGIKE EHLPHYAALPNLLAVGGSWLLQGDIAVMKKVKAAKALSP QAPG	I53-34A: 6, 8, 9, 12, 14, 22, 25, 48, 49, 50, 52, 53, 56, 73, 74, 81, 94, 95, 101, 102, 103, 104, 119, 122, 137, 140, 143, 147, 150, 151, 153, 161, 162, 163, 164, 166, 167, 170, 172, 184, 193, 198, 199, 200, 202
I53-34B SEQ ID NO: 2	(M) TKKVGIVDTTFARVDMMAAAIRTLKALSPNIKIKIRKT PGIKDLPVACKLLEEEGCDIVMALGMPGKAEKDKVCAHEA SLGLMLAQLMTNKHIIEVFVHEDEAKDDEAELDILALVRAIE HAANVYYLLFKPEYLTRMAGKGLRQGFEDAGPARE	I53-34B: 3, 12, 31, 33, 35, 36, 51, 54, 55, 56, 59, 69, 70, 71, 74, 93, 103, 106, 107, 108, 131, 132, 133, 134, 138, 142, 153
I53-40A SEQ ID NO: 3	(M) TKKVGIVDTTFARVDMASAAIILTLMESPNIKIKIRKT PGIKDLPVACKLLEEEGCDIVMALGMPGKAEKDKVCAHEA SLGLMLAQLMTNKHIIEVFVHEDEAKDDEAELKILAARRAIE HALNVYYLFKPEYLTRMAGKGLRQGFEDAGPARE	I53-40A: 3, 4, 31, 33, 35, 36, 37, 51, 54, 55, 56, 57, 59, 69, 70, 71, 74, 93, 103, 106, 118, 127, 128, 131, 132, 133, 134, 135, 138, 139, 142, 150, 153
I53-40B SEQ ID NO: 4	(M) STINNQLKALKKVPIVIAIDNAEDIIPPLGVLAENGLPA AEITFRSSAAVKAIMLLRSAQPEMLIGAGTILNGVQALAAK EAGATFVSPGFNPNTVRAQCQIIGIDIVPGVNPNSTVEAAL EMGLTTLKFFPAEASGGISMVKSLSVGPYGDIRLMPTGGITP SNIDNYLAIPQVLACGGTWMVDKKLVINGEWEDEIARLTREI VEQVNP	I53-40B: 2, 3, 7, 9, 10, 12, 20, 21, 23, 26, 27, 30, 34, 38, 45, 60, 62, 75, 85, 94, 95, 122, 124, 126, 134, 139, 143, 151, 153, 161, 163, 166, 167, 170, 172, 180, 184, 185, 186, 189, 190, 192, 193, 194, 195, 198, 201, 202, 205, 208, 209
I53-47A SEQ ID NO: 5	(M) PIFTLNTNIKATDVPSDFLSLTSRLVGLLILSKPGSYVA VHINTDQQLSFGGSTNPAAFTGLMSIGGIEPSKNRDHSAVL FDHNLAMLGIPKNRMYIHVNLNQDDVGWNGTTF	I53-47A: 11, 13, 14, 17, 34, 36, 37, 45, 47, 54, 55, 56, 65, 69, 70, 71, 74, 91, 92, 93, 101, 103, 105, 109, 110, 112, 114
I53-47B SEQ ID NO: 6	(M) NQHSHKDYETVRIAVVRARWHADIVDACVEAFEIAMA IGGDRFAVDVFDVPGAYEIPHLHARTLAETGRYGAFLGTA VNGGIYRHEFVASAVIDGMMNVQLSTGVPVLSAVLTPHRYR DSAEHHRFFAAHFAVKGVEARACIEILAAREKIAA	I53-47B: 6, 7, 8, 9, 10, 11, 13, 18, 20, 21, 24, 43, 44, 51, 63, 67, 70, 85, 87, 101, 105, 122, 123, 124, 125, 126, 147, 152, 153, 154

TABLE 2-continued

Name	Amino Acid Sequence	Surface residues not near interface
I53-50A SEQ ID NO: 7	(M) KMEELPKKKHIVAVLRLANSVEEAIKEKAVAVPAGGVHLI EITFTVDPADTVIKALSVIKEKGAIIGAGTVTSVEQCRKAV ESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMPTTELVKAM KLGHТИLKFPGEVVGPQFVKAMKGPFPMNVKFVPTGGVNL NVCEWFKAQVLAVVGGSALVKGTPDEVREKAKAPVEKIRGC TE	I53-50A: 4, 5, 6, 8, 9, 11, 17, 19, 23, 37, 46, 47, 59, 74, 77, 78, 81, 94, 95, 98, 101, 102, 103, 106, 119, 122, 126, 139, 142, 145, 149, 150, 152, 160, 161, 162, 163, 166, 169, 179, 183, 185, 188, 191, 192, 194, 198, 199
I53-50B SEQ ID NO: 8	(M) NQHSHKDYETVRIAVVRARWHAIEVDACVSAFEAAMAD IGGDRFAVDVFDVPGAYEIPHLHARTLAETGRYGAVLGTAJV VNGGIYRHEFPVASAVIDGMNNVQLSTGVPVLSAVLTPHRYR DSDAHILLFLALFAVKGMEEAACRACEVILAAREKIAA	I53-50B: 6, 7, 8, 9, 10, 11, 13, 18, 20, 21, 34, 38, 39, 40, 43, 44, 48, 51, 63, 67, 70, 87, 101, 105, 118, 143, 147, 152, 153, 154
I53-51A SEQ ID NO: 9	(M) FIKSGDDGNINVINKRKGDKSPLVNPLGDLDELNSFIG FAISKIPWEDMKKDLERVQVLFEGEDLSTQSSKKKIDES YVLWLLAATAIYRIESGPVKLFVIPGGSEEAASVLHVTRSV ARRVERNAVAKYTKEPEINRMIIVYLNRLSSLFAMALVANK RRNQSEKIKYEIGKSW	I53-51A: 19, 20, 24, 28, 46, 47, 51, 70, 71, 73, 74, 75, 76, 102, 122, 130, 133, 134, 135, 136, 137, 140, 162, 163, 164, 165, 169, 175, 177
I53-51B SEQ ID NO: 10	(M) NQHSHKDYETVRIAVVRARWHADIEVDQCVRFAEEAMAD AGGDRAVDVFDVPGAYEIPHLHARTLAETGRYGAVLGTAJV VNGGIYRHEFPVASAVIDGMNNVQLSTGVPVLSAVLTPHRYR SSREHHFFREHFMVKGVEAAAACITILAAREKIAA	I53-51B: 6, 7, 8, 9, 10, 11, 13, 18, 21, 27, 34, 38, 43, 48, 63, 67, 70, 85, 87, 101, 118, 125, 126, 129, 152, 153, 154
I52-03A SEQ ID NO: 11	(M) GHTKGPTPQQHDGSALRIGIVHARWNKTIIMPLLIGTI AKLLECGVKASNIIVQSVPGSWELPIAVQRLYSASQLQTPS SGPSLSAGDLLGSSTTDLALPTTASSTGPFDALIAIGVL IKGETMHFEYIADSVSHGLMRVQLDTGVPIFGVLTIVLDD QAKARAGVIEGSHNHGEDWGLAAVEMGVRRDWAAAGKTE	I52-03A: 6, 9, 10, 11, 13, 15, 16, 26, 48, 69, 75, 76, 78, 79, 111, 125, 127, 142, 146, 159, 160, 161, 162, 171, 175, 193, 194, 196, 197, 199, 200
I52-03B SEQ ID NO: 12	(M) YEVDHADYVDFYLGRGKDYAAEASIDIADLVRSLRTPEA SSLLDVACGTGTHLEHFTKEFGDTAGLELSEDMLTHARKRL PDATLHQGDMRDFQLGRKFSAVVSMFSSVGYLKTVAELGAA VASFAEHLEPGVVVVEPWFPETFADGVVSADVRRDGRT VARVSHSVREGNATRMVHFTVADPGKGVRHFSDVHLITLF HQREYEEAFMAAGLRVEYLEGGPSGRGLFVGVPA	I52-03B: 2, 3, 5, 6, 8, 15, 17, 20, 22, 23, 26, 27, 30, 33, 34, 35, 37, 38, 40, 54, 55, 57, 58, 59, 61, 62, 68, 70, 71, 74, 77, 78, 79, 81, 82, 84, 86, 87, 91, 96, 97, 98, 111, 127, 130, 131, 132, 141, 144, 145, 148, 150, 154, 157, 158, 159, 160, 161, 171, 172, 173, 174, 177, 187, 189, 192, 198, 199, 222, 223, 224, 236
I52-32A SEQ ID NO: 13	(M) GMKEKFVLIITHGDFGKGLLSGAEVIIKGKQENVHTVGL NLDGNIKEVAKEMRRIIAKLAEDKEIIIVVDLFGGSPNIALEMMKTFDVKVITGINMPMLVELLTSINVYDTTELENIS KIGKDGKIVIEKSSLKM	I52-32A: 3, 5, 15, 18, 30, 32, 35, 40, 41, 42, 44, 45, 65, 73, 79, 91, 103, 106, 109, 110, 111, 112, 114, 115, 118, 122, 123, 125, 126, 129, 131
I52-32B SEQ ID NO: 14	(M) KYDGSKLRLIGILHARWNLEIIAALVAGAIKRLQEFGVK AENIIIETVPGSFELPYGSKLFVKEQKRLGKPLDAIPIGV LIKGSTMHFEYICDSTTHQLMKLNFELGIPVIFGVLTCLTD EQAEARAGLIEGKMHNHGEDWGAAGEMATKFN	I52-32B: 4, 6, 7, 9, 17, 32, 35, 42, 59, 63, 64, 66, 67, 68, 69, 70, 71, 73, 83, 85, 90, 106, 119, 120, 121, 122, 125, 131, 133, 134, 135, 136, 154
I52-33A SEQ ID NO: 15	(M) AVKGLGEVDQKYDGSKLRLIGILHARWNRKII LALVAGA VLRLLEFGVKAENIIIETVPGSFELPYGSKLFVKEQKRLGK PLDAIPIGVLIKGSTMHFEYICDSTTHQLMKLNFELGIPV IFGVLTCLTDEQAEARAGLIEGKMHNHGEDWGAAGEMATKFN	I52-33A: 12, 14, 16, 17, 19, 26, 27, 46, 69, 73, 74, 76, 77, 78, 80, 81, 83, 93, 95, 100, 116, 129, 130, 131, 132, 145, 164

TABLE 2-continued

Name	Amino Acid Sequence	Surface residues not near interface
I52-33B SEQ ID NO: 16	(M) GANWYLDNESSRLSFTSTKNADIAEVHRFLVLHGKVDP KGLAEVEVETESTISTGIPLRDMLLRLVLFQVSKEPVQAQINA QLDMRPIINNLAPGAQLELRPLTVSLRGKSHSYNAELLATR LDERRFQVVTLEPLVIHAQDFDMVRFAFNALRLVAGLSAVSL SVPVGAVLIFTAR	I52-33B: 4, 6, 10, 20, 21, 23, 24, 31, 32, 34, 36, 39, 40, 42, 44, 46, 48, 56, 73, 77, 81, 83, 85, 88, 89, 91, 92, 96, 97, 99, 101, 103, 109, 110, 111, 112, 114, 124, 125, 138, 140, 143, 158, 175
I32-06A SEQ ID NO: 17	(M) TDYIRDGSAIKALSFAIIAELDLRHPQDLQLR AVR IHACGMVDVANDLAFSEGAKAGR NALLAGAPILCDARMVA EGITRSRLPADNRVITYTLSLPSVPELAKKIGNTRSAALDL WLPHIEGSIV AIGNAPTAFLRLFELLDAGAPKPAIIGMPV GFVGAAESKDELAANSRGVPYVIVRGRRGGSAM TAAVNL ASERE	I32-06A: 24, 26, 27, 41, 47, 50, 51, 56, 60, 63, 64, 67, 68, 77, 84, 85, 86, 91, 93, 98, 99, 100, 101, 102, 105, 108, 109, 114, 123, 124, 125, 127, 135, 142, 145, 148, 149, 152, 153, 169, 172, 173, 176, 177, 180, 187, 189
I32-06B SEQ ID NO: 18	(M) ITVFGLKSKLAPRREKLAEVIVSSLHLGLDIPKGKHAI RFLCLEKEDFYYPFDRSDDYT VIEINLMAGRSEETKMLLIF LLFIALERKLGI RAHDV EITIKEQPAHCWGFRGRTGDSARD LDYDIYV	I32-06B: 8, 9, 10, 13, 14, 15, 16, 17, 20, 34, 36, 45, 46, 47, 50, 51, 53, 54, 57, 67, 70, 91, 93, 95, 105, 112
I32-19A SEQ ID NO: 19	(M) GS DLQKLQR FSTCDISDG LNNV YPIGGYFPN LT A IS PPQN SSI VG TAYT VTL FAPI DPPR PAVN YIDS VPP NS I L VLA LEPHLQS QFHPFI KIT QAM YGGL MSTR A QYL KSN GTV VFGR IRDV DEH RTL NH PVP FAY GVGSCAP KAVV KAV GTN VQL KLT SDG VTQ TIC P GDPY IAGDN NGIV R I P V QET DIS KLV TYIEKS IEV DRL VSE AIK NGL PAK AA QTARR MVL KDYI	I32-19A: 3, 4, 6, 7, 9, 10, 25, 27, 36, 40, 42, 43, 44, 49, 58, 59, 61, 62, 63, 70, 72, 73, 74, 82, 84, 88, 89, 109, 110, 112, 126, 127, 129, 130, 132, 146, 155, 156, 157, 159, 166, 169, 172, 189, 190, 192, 194, 195, 198, 201, 204, 215, 232
I32-19B SEQ ID NO: 20	(M) SGMRVYL GADHAG YELK QAI IAFL KMTG HEP IDC GAL R YD ADDD YP AFC CAA AAT RTV AD PG SLG I VL GGSG NGE QIA AN KVP GARC ALA WSV QTA ALA REH HNA QOL I GIG GR MHT L EAL RIV KAF VTT PWSKA QRH QRR IDIL A EYER THE APP VPG APA	I32-19B: 4, 5, 31, 33, 38, 41, 42, 43, 55, 56, 59, 61, 62, 83, 93, 94, 101, 104, 113, 119, 129, 131, 134, 136, 137, 139, 140, 143, 144, 146, 147, 150, 152, 153, 156, 158, 159
I32-28A SEQ ID NO: 21	(M) GDDARIAAIGDVDELNSQIGVLLAEP LPDDVRAA LSA I QHDLFDLGELCIPGHAAITEDHLLRLA L WL VHYNGQLPPL EEFILPGGARGAALAHVCRTVCRR AERSIKALGASEPLNIA PAAYVNLLSDLLFVLARVLNRAAGGADVLWDRTRAH	I32-28A: 4, 6, 7, 10, 14, 27, 30, 31, 33, 34, 41, 44, 45, 51, 52, 53, 54, 55, 56, 59, 76, 78, 79, 80, 81, 82, 83, 90, 103, 111, 115, 116, 131, 134, 142, 145, 147, 150
I32-28B SEQ ID NO: 22	(M) IL SAEG QFT L RHP HGQAA AL AFV REPA A AL AGV QRL RG L DSD GEQ V WGE LL VR VPL VGE DLP FR SE I V RTP QGA EL RP L TL TGERA NVA VSG QATA AEGG EMA FAF QF QAH L AT PEA EG EGG AAF EVM VQAA AGV TLL VAM ALP QG L AAGL PPA	I32-28B: 3, 4, 6, 8, 12, 15, 17, 18, 22, 26, 28, 32, 38, 39, 41, 43, 45, 46, 48, 50, 60, 66, 68, 71, 73, 74, 79, 81, 82, 83, 84, 86, 87, 95, 100, 103, 105, 109, 111, 113, 151, 152, 155, 156, 157
I53-40A.1 SEQ ID NO: 23	(M) T KKVGIVDTT F ARV DMAS AAI TLK MESP NIKI IRKT V PGIKDLPVACKL LEEEGCDIVM ALGMP GKKE DKV CAHEA SLGLMLAQLMTNKH IIEV FV HEDE AK DDAEL KILA ARRA IE HALNVYYLLFKPEYLTRMAGKGLRQGFEDAGPARE	I53-40A: 3, 4, 31, 33, 35, 36, 37, 51, 54, 55, 56, 57, 59, 69, 70, 71, 74, 93, 103, 106, 118, 127, 128, 131, 132, 133, 134, 135, 138, 139, 142, 150, 153
I53-40B.1 SEQ ID NO: 24	(M) DDINNQKL RL KVPIVIAIDNAE D II PLG KVLA ENGL PA AEITF RSS AAV KAI MLL RLSA QP EMLI GAGT IL NGV QAL A K EAGADF VVSPG FNPT VR ACQI I GIDIV PGV NN P STV EQA L EMGL TTL KF PP AEASGG I S M V KSL VGP YGD I RLM P T G G I TP DNID NYLA I P QV LACGG TWM VDK L V R NGE WDE I ARL T RE I VEQ VNP	I53-40B: 2, 3, 7, 9, 10, 12, 20, 21, 23, 26, 27, 30, 34, 38, 45, 60, 62, 75, 85, 94, 95, 122, 124, 126, 134, 139, 143, 151, 153, 161, 163, 166, 167, 170, 172, 180,

TABLE 2-continued

Name	Amino Acid Sequence	Surface residues not near interface
I53-47A.1 SEQ ID NO: 25	(M) PIPTLNNTNIKADDVPSDFLSLTSRLVGLILSKPGSYVA VHINTDQQLSFGGSTNPAAFGTLMSIGGIEPDKNRDHSAVL FDHLNAMLGIPKPNRMYIHFVNLngDDVGWNGTTF	184, 185, 186, 189, 190, 192, 193, 194, 195, 198, 201, 202, 205, 208, 209
I53-47A.1NegT2 SEQ ID NO: 26	(M) PIPTLNNTNIKADDVPSDFLSLTSRLVGLILSEPGSYVA VHINTDQQLSFGGSTNPAAFGTLMSIGGIEPDKNEDHSAVL FDHLNAMLGIPKPNRMYIHFVDLDGVNGTTF	I53-47A: 11, 13, 14, 17, 34, 36, 37, 45, 47, 54, 55, 56, 65, 69, 70, 71, 74, 91, 92, 93, 101, 103, 105, 109, 110, 112, 114
I53-47B.1 SEQ ID NO: 27	(M) NQHSHKDHETVRIAVVRARWHADIVDACVEAFIAMA IGGDRFAVDVEDVPGAYEIPHLHARTLAETGRYGAVLGTA VNGGIYRHEFVASAVIDGMNNVQLDTGVGVPLSAVLTPHRY DSDEHHRFFAAHFAVKGVVEARACIEILNAREKIAA	I53-47A: 11, 13, 14, 17, 34, 36, 37, 45, 47, 54, 55, 56, 65, 69, 70, 71, 74, 91, 92, 93, 101, 103, 105, 109, 110, 112, 114
I53-47B.1NegT2 SEQ ID NO: 28	(M) NQHSHKDHETVRIAVVRARWHADIVDACVEAFIAMA IGGDRFAVDVEDVPGAYEIPHLHARTLAETGRYGAVLGTA VDGGIYDHEFVASAVIDGMNNVQLDTGVGVPLSAVLTPH YE DSDEDHEFFAAHFAVKGVVEARACIEILNAREKIAA	I53-47B: 6, 7, 8, 9, 10, 11, 13, 18, 20, 21, 24, 43, 44, 51, 63, 67, 70, 85, 87, 101, 105, 122, 123, 124, 125, 126, 147, 152, 153, 154
I53-50A.1 SEQ ID NO: 29	(M) KMEELFKHKIVAVLTRANSVEEAIEKAVAVFAGGVH LIEITFTVPDADTVIKALSVLK ESGAEFIVSPHLDEEISQFC KLGDILKLFPGEVVGPEF NVCEWFKAGVLAVGVGDALV TE	I53-50A: 4, 5, 6, 8, 9, 11, 17, 19, 23, 37, 46, 47, 59, 74, 77, 78, 81, 94, 95, 98, 101, 102, 103, 106, 119, 122, 126, 139, 142, 145, 149, 150, 152, 160, 161, 162, 163, 166, 169, 179, 183, 185, 188, 191, 192, 194, 198, 199
I53-50A.1NegT2 SEQ ID NO: 30	(M) KMEELFKHKIVAVLTRANSVEEAIEKAVAVFAGGVH LIEITFTVPDADTVIKALSVLK ESGAEFIVSPHLDEEISQFC KLGDILKLFPGEVVGPEF DVCEWFDA TE	I53-50A: 4, 5, 6, 8, 9, 11, 17, 19, 23, 37, 46, 47, 59, 74, 77, 78, 81, 94, 95, 98, 101, 102, 103, 106, 119, 122, 126, 139, 142, 145, 149, 150, 152, 160, 161, 162, 163, 166, 169, 179, 183, 185, 188, 191, 192, 194, 198, 199
I53-50A.1PostT1 SEQ ID NO: 31	(M) KMEELFKHKIVAVLTRANSVEEAIEKAVAVFAGGVH LIEITFTVPDADTVIKALSVLK ESGAEFIVSPHLDEEISQFC KLGDILKLFPGEVVGPEF NVCKWFKAGVLAVGVGK TE	I53-50A: 4, 5, 6, 8, 9, 11, 17, 19, 23, 37, 46, 47, 59, 74, 77, 78, 81, 94, 95, 98, 101, 102, 103, 106, 119, 122, 126, 139, 142, 145, 149, 150, 152, 160, 161, 162, 163, 166, 169, 179, 183, 185, 188, 191, 192, 194, 198, 199
I53-50B.1 SEQ ID NO: 32	(M) NQHSHKDHETVRIAVVRARWHAEIVDACVS IGGDRFAVDVEDVPGAYEIPHLHARTLAETGRYGA VNGGIYRHEFVASAVIDGM DSDAHILLFLALFAVK TE	I53-50B: 6, 7, 8, 9, 10, 11, 13, 18, 20, 21, 34, 38, 39, 40, 43, 44, 48, 51, 63, 67, 70, 87, 101, 105, 118, 143, 147, 152, 153, 154
I53-50B.1NegT2 SEQ ID NO: 33	(M) NQHSHKDHETVRIAVVRARWHAEIVDACVS IGGDRFAVDVEDVPGAYEIPHLHARTLAETGRYGA VDGGIYDHEFVASAVIDGM DSDADTLLFLALFAVK TE	I53-50B: 6, 7, 8, 9, 10, 11, 13, 18, 20, 21, 34, 38, 39, 40, 43, 44, 48, 51, 63, 67, 70, 87, 101, 105, 118, 143, 147, 152, 153, 154
I53-50B.1PostT1 SEQ ID NO: 34	(M) NQHSHKDHETVRIAVVRARWHAEIVDACVS IGGDRFAVDVEDVPGAYEIPHLHARTLAETGRYGA VNGGIYRHEEVASAVING KSKAHTLLFLALFAVK TE	I53-50B: 6, 7, 8, 9, 10, 11, 13, 18, 20, 21, 34, 38, 39, 40, 43, 44, 48, 51, 63, 67, 70, 87, 101, 105, 118, 143, 147, 152, 153, 154

- [0061] In various embodiments of the nanostructure of the invention, the first polypeptides and the second polypeptides comprise polypeptides with the amino acid sequence selected from the following pairs, or modified versions thereof (i.e.: permissible modifications as disclosed for the polypeptides of the invention: isolated polypeptides comprising an amino acid sequence that is at least 75% 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical over its length, and/or identical at least at one identified interface position, to the amino acid sequence indicated by the SEQ ID NO.):
- [0062] SEQ ID NO:1 and SEQ ID NO:2 (I53-34A and I53-34B);
[0063] SEQ ID NO:3 and SEQ ID NO:4 (I53-40A and I53-40B);
[0064] SEQ ID NO:3 and SEQ ID NO:24 (I53-40A and I53-40B.1);
[0065] SEQ ID NO:23 and SEQ ID NO:4 (I53-40A.1 and I53-40B);
[0066] SEQ ID NO:35 and SEQ ID NO:36 (I53-40A genus and I53-40B genus);
[0067] SEQ ID NO:5 and SEQ ID NO:6 (I53-47A and I53-47B);
[0068] SEQ ID NO:5 and SEQ ID NO:27 (I53-47A and I53-47B.1);
[0069] SEQ ID NO:5 and SEQ ID NO:28 (I53-47A and I53-47B.1NegT2);
[0070] SEQ ID NO:25 and SEQ ID NO:6 (I53-47A.1 and I53-47B);
[0071] SEQ ID NO:25 and SEQ ID NO:27 (I53-47A.1 and I53-47B.1);
[0072] SEQ ID NO:25 and SEQ ID NO:28 (I53-47A.1 and I53-47B.1NegT2);
[0073] SEQ ID NO:26 and SEQ ID NO:6 (I53-47A.1NegT2 and I53-47B);
[0074] SEQ ID NO:26 and SEQ ID NO:27 (I53-47A.1NegT2 and I53-47B.1);
[0075] SEQ ID NO:26 and SEQ ID NO:28 (I53-47A.1NegT2 and I53-47B.1NegT2);
[0076] SEQ ID NO:37 and SEQ ID NO:38 (I53-47A genus and I53-47B genus);
[0077] SEQ ID NO:7 and SEQ ID NO:8 (I53-50A and I53-50B);
[0078] SEQ ID NO:7 and SEQ ID NO:32 (I53-50A and I53-50B.1);
[0079] SEQ ID NO:7 and SEQ ID NO:33 (I53-50A and I53-50B.1NegT2);
[0080] SEQ ID NO:7 and SEQ ID NO:34 (I53-50A and I53-50B.4PosT1);
[0081] SEQ ID NO:29 and SEQ ID NO:8 (I53-50A.1 and I53-50B);
[0082] SEQ ID NO:29 and SEQ ID NO:32 (I53-50A.1 and I53-50B.1);
[0083] SEQ ID NO:29 and SEQ ID NO:33 (I53-50A.1 and I53-50B.1NegT2);
[0084] SEQ ID NO:29 and SEQ ID NO:34 (I53-50A.1 and I53-50B.4PosT1);
[0085] SEQ ID NO:30 and SEQ ID NO:8 (I53-50A.1NegT2 and I53-50B);
[0086] SEQ ID NO:30 and SEQ ID NO:32 (I53-50A.1NegT2 and I53-50B.1);
[0087] SEQ ID NO:30 and SEQ ID NO:33 (I53-50A.1NegT2 and I53-50B.1NegT2);

- [0088] SEQ ID NO:30 and SEQ ID NO:34 (I53-50A.1NegT2 and I53-50B.4PosT1);
[0089] SEQ ID NO:31 and SEQ ID NO:8 (I53-50A.1PosT1 and I53-50B);
[0090] SEQ ID NO:31 and SEQ ID NO:32 (I53-50A.1PosT1 and I53-50B.1);
[0091] SEQ ID NO:31 and SEQ ID NO:33 (I53-50A.1PosT1 and I53-50B.1NegT2);
[0092] SEQ ID NO:31 and SEQ ID NO:34 (I53-50A.1PosT1 and I53-50B.4PosT1);
[0093] SEQ ID NO:39 and SEQ ID NO:40 (I53-50A genus and I53-50B genus);
[0094] SEQ ID NO:9 and SEQ ID NO:10 (I53-51A and I53-51B);
[0095] SEQ ID NO:11 and SEQ ID NO:12 (I52-03A and I52-03B);
[0096] SEQ ID NO:13 and SEQ ID NO:14 (I52-32A and I52-32B);
[0097] SEQ ID NO:15 and SEQ ID NO:16 (I52-33A and I52-33B);
[0098] SEQ ID NO:17 and SEQ ID NO:18 (I32-06A and I32-06B);
[0099] SEQ ID NO:19 and SEQ ID NO:20 (I32-19A and I32-19B);
[0100] SEQ ID NO:21 and SEQ ID NO:22 (I32-28A and I32-28B);
[0101] SEQ ID NO:23 and SEQ ID NO:24 (I53-40A.1 and I53-40B.1);
[0102] SEQ ID NO:41 and SEQ ID NO:42 (T32-28A and T32-28B);
[0103] SEQ ID NO:43 and SEQ ID NO:44 (T33-09A and T33-09B);
[0104] SEQ ID NO:45 and SEQ ID NO:46 (T33-15A and T33-15B);
[0105] SEQ ID NO:47 and SEQ ID NO:48 (T33-21A and T33-21B);
[0106] SEQ ID NO:49 and SEQ ID NO:50 (T33-28A and T32-28B); and
[0107] SEQ ID NO:51 and SEQ ID NO:44 (T33-31A and T33-09B (also referred to as T33-31B))

[0108] In one embodiment, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, are expressed as a fusion protein with the first and/or second polypeptides. In these embodiments, it is preferred that the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof are present at the N terminus of the fusion protein, whenever this configuration can facilitate presentation of the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof on an exterior of the nanostructure. This preference for the presence of the paramyxovirus and/or pneumovirus F protein at the N terminus of the fusion protein derives from the location of the C terminus of the paramyxovirus and/or pneumovirus F proteins at one extreme (the “bottom”) of the F protein trimer; by locating the genetic fusion at this point, the majority of the F protein structure will be displayed and accessible on the nanostructure exterior. In a further embodiment, the nanostructures comprise one or more copies of a fusion protein comprising at least two domains—a paramyxovirus and/or pneumovirus F protein, or an antigenic fragment thereof, and a trimeric assembly domain (i.e.: each first assembly is a homotrimer of the first polypeptide)—and one or more copies of a second oligomeric block (i.e.: each second assembly is an oligomer of two or

more copies of the second polypeptide). In another embodiment, the first and/or second polypeptides may be modified to permit the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, to be covalently linked to the first and/or second polypeptides. In one non-limiting example, the first and/or second polypeptides can be modified, such as by introduction of various cysteine residues at defined positions to facilitate linkage one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof.

[0109] In other embodiments, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof are attached to the first or second polypeptides via any suitable technique, including but not limited to covalent chemical cross-linking (via any suitable cross-linking technique) and non-covalent attachment including engineered electrostatic interactions.

Trimeric Assembly Domains

[0110] In one embodiment of a trimeric assembly that comprises a trimeric paramyxovirus and/or pneumovirus F protein, or antigenic fragments thereof, the paramyxovirus and/or pneumovirus F protein, or antigenic fragment thereof is genetically fused to the first polypeptides that self-assemble into the trimeric assembly. The trimeric assembly comprises a protein-protein interface that induces three copies of the first polypeptides to self-associate to form trimeric building blocks. Each copy of the first polypeptides further comprises a surface-exposed interface that interacts with a complementary surface-exposed interface on a second assembly domain. As described in King et al. (Nature 510, 103-108, 2014), Bale et al. (Science 353, 389-394, 2016), and patent publications WO2014124301 A1 and US20160122392 A1, the complementary protein-protein interface between the trimeric assembly domain and second assembly domain drives the assembly of multiple copies of the trimeric assembly domain and second assembly domain to a target nanostructure. In some embodiments, each copy of the trimeric assembly domains of the nanostructure bears a paramyxovirus and/or pneumovirus F proteins, or antigenic fragment thereof, as a genetic fusion; these nanostructures display the F proteins at full valency. In other embodiments, the nanostructures of the invention comprise one or more copies of trimeric assembly domains bearing paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof as genetic fusions as well as one or more trimeric assembly domains that do not bear F proteins as genetic fusions; these nanostructures display the F proteins at partial valency. The trimeric assembly domain can be any polypeptide sequence that forms a trimer and interacts with a second assembly domain to drive assembly to a target nanostructure.

[0111] In one specific embodiment, the first 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 T33-31A (SEQ ID NO:51) 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 T33-09B/T33-31B (SEQ ID NO:44) (residues in parentheses are optional)

T33-31A

(SEQ ID NO: 51)

(M) EEVVLITVPSALVAKIAHALVEERLAACVNIVPGLTSIYREEGSV

VSDHELLLKVITTDAPPKLKERVKELHPYEVPEIVALPIAEGNREYLD

WLRENTG

>T33-31B

(SEQ ID NO: 44)

(M) VRGIRGAITVEEDTPAAILAATIELLLKMLEANGIQSYEELAAVIF

TVTEDLTSAFPAEAARLIGMHRVPLLSAREVPVPGSLPRVIRVLALWNT

DTPQDRVRHVYLNEAVRLRPDLESAQ

[0112] In another specific embodiment, the first 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 T33-15A (SEQ ID NO:45) 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 T33-15B (SEQ ID NO:46).

[0113] In various further specific embodiments, the first 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 a polypeptides selected from the group consisting of I53-50A (SEQ ID NO:7), I53-50A.1 (SEQ ID NO:29), I53-50A.1NegT2 (SEQ ID NO:30), and I53-50A.1PosT1 (SEQ ID NO:31), 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 a polypeptide selected from the group consisting of I53-50B (SEQ ID NO:8), I53-50B.1 (SEQ ID NO:32), I53-50B.1NegT2 (SEQ ID NO:33), and I53-50B.4PosT1 (SEQ ID NO:34).

[0114] In another specific embodiment, the first 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 I32-28A (SEQ ID NO:21) 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 I32-28B (SEQ ID NO:22).

[0115] The nanostructures of the invention display multiple copies (i.e.: 2, 3, or more) of one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, on an exterior of the nanostructure. Exemplary paramyxovirus and/or pneumovirus include, but are not limited to, respiratory syncytial virus (RSV) and Human metapneumovirus (hMPV). (C. L. Afonso et al., Taxonomy of the order Mononegavirales: update 2016. Arch. Virol. 161, 2351-2360 (2016)).

[0116] As used herein, “on an exterior of the nanostructure” means that an antigenic portion of the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, must be accessible for binding by B cell receptors, antibodies, or antibody fragments and not buried within the nanostructure.

[0117] The one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, may com-

prise any suitable native F proteins, post-fusion, or pre-fusion (preF) antigens, or mutants thereof capable of inducing an immune response that will generate antibodies that bind to paramyxovirus and/or pneumovirus F proteins. A nanostructure may display more than one F protein; thus, in some embodiments the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof comprise 1, 2, 3, 4, or more F proteins or antigenic fragments thereof. In one embodiment, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof may be as defined in patent publication number US 2016/0046675 A1. In some embodiments, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, are selected from the group consisting of SEQ ID NOS: 1-350, 370-382, 389-693, 698-1026, 1429-1442, 1456-1468, and 1474-1478 as disclosed in US published patent application 2016/0046675. In other embodiments, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof may be as defined in WO2012158613, US 20160102123, US20140141037, WO2014079842, WO2014160463, US20140271699, EP2970393, WO2014174018, US20140271699, US20160176932, US20160122398, WO2017040387, WO2017109629, WO2017172890, WO2017207477, Krarup et al. (2015) *Nature Communications* 6:8143, and WO2017207480.

[0118] In a specific embodiment, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, comprise a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to the amino acid sequence of DS-Cav1 shown below (residues in parentheses are optional; note that the N-terminal residues in parentheses are cleaved from the protein during secretion—the mature N terminus begins with QNITEEF . . . (SEQ ID NO:52)). DS-Cav1 comprises a prefusion-stabilized form of the fusion (F) glycoprotein, which elicits improved protective responses against respiratory syncytial virus (RSV) in mice and macaques compared to postfusion RSV F (McLellan et al. (2013) *Science* 342:592-8).

DS-Cav1 (SEQ ID NO: 53) :
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLSA
 LRTGWYTsvitieLSNIKENCNGTDAVKLIQELDKYKNAVTELQLL
 MQSTPATNNRARRELPRFMNYTLNNAKTKTNVTLSKKRKRFLGFLLGVG
 SAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVLD
 LKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGV
 TTPVSTYMLTNSELLSINDMPITNDQKKLMSNNVQIVRQQSYSIMCI
 KEEVLAYVVLQLPLYGVIDTPCWKLHTSPLCNTNTKEGSNICLRTDRGW
 YCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTPSEVNLCNVDI FNPK

-continued
 YDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIIKTFSNGC
 DYVSNKGVDTSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSEDF
 DASISQVNEKINQSLAFIR (KSDELL)

[0119] In other embodiments, the F protein may comprise a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to a polypeptide selected from:

RSV F
 sc9-10 DS-Cav1 A149C Y458C
 (SEQ ID NO: 61)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLSA
 LRTGWYTsvitieLSNIKENCNGTDAVKLIQELDKYKNAVTELQLL
 MQSTPATGSGSAICSGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNG
 VSVLTFKVLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEI
 TREFSVNAVGVTTPVSTYMLTNSELLSINDMPITNDQKKLMSNNVQIVR
 QQSYSIMCIKEEVLAYVVLQLPLYGVIDTPCWKLHTSPLCNTNTKEGSN
 ICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSRTLPS
 LCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKRN
 GIICKTFSNGCDYVSNKGVDTSVGNTLYCVNKQEGKSLYVKGEPIINFY
 DPLVFPSEDFDASISQVNEKINQSLAFIR (KSDELL)

sc9-10 DS-Cav1 A149C Y458C S46G K465Q S215P E92D
 (SEQ ID NO: 62)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLG
 LRTGWYTsvitieLSNIKENCNGTDAVKLIQELDKYKNAVTDLQLL
 MQSTPATGSGSAICSGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNG
 VSVLTFKVLDLKNYIDKQLLPILNKQSCSIPNIEFQQKNNRLLEI
 TREFSVNAVGVTTPVSTYMLTNSELLSINDMPITNDQKKLMSNNVQIVR
 QQSYSIMCIKEEVLAYVVLQLPLYGVIDTPCWKLHTSPLCNTNTKEGSN
 ICLTRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSRTLPS
 LCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKRN
 GIICKTFSNGCDYVSNKGVDTSVGNTLYCVNKQEGQSLYVKGEPIINFY
 DPLVFPSEDFDASISQVNEKINQSLAFIR (KSDELL)

[0120] SEQ ID NO:61-62 represent second-generation stabilized DS-Cav immunogens; mutations relative to DS-Cav1 are noted and it should be noted that the present disclosure contemplates the use of DS-Cav1 mutants that differ by a single one of the noted amino acid substitutions in SEQ ID NO:61 or 62 above, or two or more of the amino acid substitutions noted. In other embodiments, the F protein may comprise one or more of the following, each of which may additionally include 1, 2, or more of the noted amino acid substitutions in SEQ ID NO:61 or 62 above:

RSV F SC-DM (N67I, S215P)
 (SEQ ID NO: 63)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLSALRTGWYTsvitieLSN
 IKKIKCNGTDAKIKLIKQELDKYKNAVTELQLLMQSTPATNNQARGSGSGRSLGFLGVGSIAAS

- continued

GVAVSKVLHLEGEVNKIKSALLSTNKAVVSLNSNGSVLTSKVLNLKNYIDKQLLPVNQKSCSIP
 NIETVIEFQQKNNRLLITREFSVNAGVTPVSTYMLTNSELLSINDMPITNDQKKLMSNNVQI
 VRQQSYSIMSIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCCTNTKEGSNICLRTDRGWYCDN
 AGSVSFFPQAETCKVQSNRVCDFTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITS
 GAIIVSCYGKTKTASNKNRGIKTFNSNGCDYVSNKGVDTSVGNTLYYVNKQEGKSLYVKGEPII
 NFYDPLVFPSPDEFDASISQVNEKINQSLAFIR (KSDELLSAIGGYIPEAPRDGQAYVRKDGEWVL
LSTFL)

SC-TM (N671, S215P, and E487Q)

(SEQ ID NO: 64)

(MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYSALRTGWTTSVITIELSN
 IKKIKCNGTDAKIKLIKQELDKYKNAVTELQLLMQSTPATNNQARGSGSGRSLGFLGVGSAIAS
 GVAVSKVLHLEGEVNKIKSALLSTNKAVVSLNSNGSVLTSKVLNLKNYIDKQLLPVNQKSCSIP
 NIETVIEFQQKNNRLLITREFSVNAGVTPVSTYMLTNSELLSINDMPITNDQKKLMSNNVQI
 VRQQSYSIMSIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCCTNTKEGSNICLRTDRGWYCDN
 AGSVSFFPQAETCKVQSNRVCDFTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITS
 GAIIVSCYGKTKTASNKNRGIKTFNSNGCDYVSNKGVDTSVGNTLYYVNKQEGKSLYVKGEPII
 NFYDPLVFPSPDQFDASISQVNEKINQSLAFIR (KSDELLSAIGGYIPEAPRDGQAYVRKDGEWVL
LSTFL)

HMPV F protein, strain CAN97-83 (A2)

(SEQ ID NO: 65)

(MSWKVVIIIFSLLITPQHG) LKESYLEECSIITEGYSVLRTGWTNVFTLEVGDVENLICSDG
 PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRFVLGAIALGVATAAAVTAGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSNDAGITPAISLDMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
 LIGVYGSVIVMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWYCQAGSTVYPNE
 KDCETRGDHVFCDTAAGINVABQSKECNINISTTNYPCKVSTGRHPISMVALSPLAGLVACYKGV
 SCSIGSNRVGIIKQLNKGCYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNIG

HMPVF with A113C, A339C, T160F, I177L

(SEQ ID NO: 66)

(MSWKVVIIIFSLLITPQHG) LKESYLEECSIITEGYSVLRTGWTNVFTLEVGDVENLICSDG
 PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRFVLGAIALGVCTAAAVTAGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLAFAVRELKDFVSKNLTRALNKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSNDAGITPAISLDMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
 LIGVYGSVIVMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWYCQAGSTVYPNE
 KDCETRGDHVFCDTAAGINVABQSKECNINISTTNYPCKVSTGRHPISMVALSPLAGLVACYKGV
 SCSIGSNRVGIIKQLNKGCYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNIG

HMPV F with A113C, A120C, A339C, T160F, I177L, and Q426C

(SEQ ID NO: 67)

(MSWKVVIIIFSLLITPQHG) LKESYLEECSCTITEGYSVLRTGWTNVFTLEVGDVENLICSDG
 PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRFVLGAIALGVCTAAAVTCGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLAFAVRELKDFVSKNLTRALNKNKCDIDDLKMAVSF

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SQFNRRFLNVVRQFSNDAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
LIGVYGGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWCQNAGSTVYPNE
KDCETRGDHVFCDTACGINAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGV
SCSIGSNRVGIIKQLNKGSYITNQDADTVTIDNTVYCLSKEGEQHVIKGRPVSSSFDPKFPE
DQFNVALDQVFESIENSQALVDQSNRILSSAEKGNI

HMPV F _>AAK62968.2 fusion protein [Human metapneumovirus]
(SEQ ID NO: 101)
(MSWKVVIIIFSLITPQHG) LKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVDVENLICADG

PSLIKTELDLIKSALRELRIVSADQLAREEQIENPRQSRFVLGAIALGVATAAAVTAGVIAKTI
RLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLKMAVSF
SQFNRRFLNVVRQFSNDAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGF
LIGVYGGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWCQNAGSTVYPNE
KDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGV
SCSIGSNRVGIIKQLNKGSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKFPE
DQFNVALDQVFESIENSQALVDQSNRILSSAEKGNI

115-BV (A185P)
(SEQ ID NO: 68)
(MSWKVVIIIFSLITPQHG) LKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVDVENLICADG

PSLIKTELDLIKSALRELRIVSADQLAREEQIENPRRRRFVLGAIALGVATAAAVTAGVIAKTI
RLESEVTAIKNALKKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSF
SQFNRRFLNVVRQFSNDAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
LIGVYGGSSVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWCQNAGSTVYPNE
KDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGV
SCSIGSNRVGIIKQLNKGSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPVKFPE
DQFNVALDQVFESIENSQALVDQSNRILSSAEKGNT (SGRENLYFQGGGGSGYIPEAPRDGQAYV
RKDG EWVLLSTFLGGIEGRHHHHHH)
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[0121] In other embodiments, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, may comprise a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to an RSV F protein or mutant thereof selected from the group consisting of SEQ ID NO:53 and 61-64, wherein the polypeptide includes one or more of the following residues: 67I, 149C, 458C, 46G, 465Q, 215P, 92D, and 487Q.

[0122] In other embodiments, the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, may comprise a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity along its full length to an MPV F protein or mutant thereof selected from the group consisting of SEQ ID NO:65-68 and 101, wherein the polypeptide includes one or more of the following residues: 113C, 120C, 339C, 160F, 177L, 185P, and 426C.

Linker Between F Proteins and Trimeric Assembly Domains and Geometric Requirements

[0123] In the nanostructures of the invention, the F protein and the trimeric assembly domain may be genetically fused such that they are both present in a single polypeptide.

Preferably, the linkage between the F protein and the trimeric assembly domain allows the F protein, or antigenic fragment thereof, to be displayed on the exterior of the nanostructures of the invention. As such, the point of connection to the trimeric assembly domain should be on the exterior of the nanostructure formed by the trimeric assembly domain and the second assembly domain in the absence of any F protein. As will be understood by those of skill in the art, a wide variety of polypeptide sequences can be used to link the paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof and the trimeric assembly domain. These polypeptide sequences are referred to as linkers. Any suitable linker can be used; there is no amino acid sequence requirement to serve as an appropriate linker. There is no requirement that the linker impose a rigid relative orientation of the F protein or antigenic fragment thereof to the trimeric assembly domain beyond enabling the F protein or antigenic fragment thereof to be displayed on the exterior of the nanostructures of the invention. In some embodiments, the linker includes additional trimerization domains (e.g., the foldon domain of T4 fibritin) that assist in stabilizing the trimeric form of the F protein.

T4 fibritin foldon domain (optional in the linker region) (SEQ ID NO: 54)
GYIPEAPRDOGAYVRKDGEWVLLSTFL

[0124] In other embodiments, the linker may comprise a Gly-Ser linker (i.e.: a linker consisting of glycine and serine residues) of any suitable length. In various embodiments, the Gly-Ser linker may be 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids in length. In various embodiments, the Gly-Ser linker may comprise or consist of the amino acid sequence of GSGGSGSGGGSGSG (SEQ ID NO:55), GGSGGGSGS (SEQ ID NO:56) or GSGGSGSG (SEQ ID NO:57).

[0125] In further embodiments the linker may comprise a helical extension domain that may serve to extend the N-terminal helix of the first polypeptide, when expressed as a fusion polypeptide with the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, so that it is located at the exterior of the nanostructure surface. The helical extension may be present in combination with the other linker components described herein, or may be absent. The helical extension may be of any suitable length (i.e.: 7, 8, 9, 10, 11, 12, or more amino acids) and comprise any suitable primary amino acid sequence. In one embodiment, the helical extension may comprise or consist of the amino acid sequence EKAAKAEEAAR (SEQ ID NO:58).

[0126] Thus, in various non-limiting embodiments in which the F protein is present as a fusion protein with the first polypeptide and a linker is used, the F protein-linker sequence may comprise the following (exemplified by DS-

Cav1 as the F protein in these non-limiting embodiments). Residues in parentheses are optional and the amino acid sequence MELLILKANA^ITTILTAVTFCFASG (SEQ ID NO:59) represents the N-terminal DS-Cav1 signal peptide that is cleaved during processing:

DS-Cav1-foldon (SEQ ID NO: 60) :
(MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLSA
LRTGWYTSVITIELSNIKENKCNGTDAVKLIKQELDKYKNAVTELQLL
MQSTPATNNRARRELPRFMNTLNNAKKTNVTLSKKRKRFLGFLLGVG
SAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLSGVSVLTFKVLD
LKNYIDKQLLPILNKQSCSISNIETVIEFQQKNRRLTEITREFSVNAGV
TTPVSTYMLTNSELLSLINDMPITNDQKKLMSNNVQIVRQOQSYSIMCII
KEEVILAYVVQLPLYGVIDTPCWKLHTSPLCTINTKEGSNICLRTDRGW
YCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPESEVNLNCNVDIFNPK
YDCKIMTSKTDVSSSVITSLGAIIVSCYGKTKCTASNKRGIKTFNSNGC
DYVSNKGVDTVSVGNTLYYVNKQEGKSLYVKGEPIINFYDPLVFPSSDEF
DASISQVNEKINQSLAFIRKSDELLGYIPEAPRDGQAYVRKDGEWVLLS

[0127] In various further embodiments, the first polypeptides comprise or consist of fusion polypeptides of first polypeptides fused to an F protein, where the fusion protein has a sequence selected from the following (optional residues in parentheses):

DS-Cav1-foldon-T33-31A (SEQ ID NO: 69)

I KENKCNGTDAVKVLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKKTNVT
LSKKRKRRLFGFLLGVGSAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSGVSVLTPKVL
DLKNYIDKQLLPILNQKCSISNIETVIEFOOKNNRLLEITREFSVNAGVTTPVSTYMLTNSELL
SLINDMPITNDQKKLMSNNQIVRQQSYSIMCIKEEVLAYVQLPLYGVIDTPCWKLHTSPLC
INTKEGSNICLRTDGRGWYCDNAGSVSFFFPAETCKVQSNRVFCDTMNSLTLPSLEVNLNCNDIFN
PKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTCTASNKRGIKTFNSNGCDYVSNKGVDTVSG
NTLYYVNQEGKSLYVKGPIINFYDPLVFFSDEFDASISQVNEKINQSLAFIRKSDELLGYIPE
APRDGQAYVRKDGEWVLLSTFLGGSMEVVЛИTVPSALVAVKIAHALVEERLAACVNIVPGLTSI
YREEGSVVSIDHELLLVLVKITTDAPPKLKERVKELHPYEVPEIVALPIAEGNRREYLDWLRENTG

DS-Cav1-T33-31A
(SEQ ID NO: 70)
(MELLILKANVIATILTAVTFCFASS) QNITEEFYQSTCSAVSKGYLSALRTGWYTsvitielsn
I KENKCNGTDAKVKLIKQELDKYKNAVTELQLLMQSTPATMNRRARRELPRFMNYTLNNAAKTNVT
LSKKRKRRFLGFLLGVGSAIASGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGSVSLTFKVL
DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLETREFSVNAGVTPVSTYMLTNSELL
SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIKEEVLAYVQPLYGVIDTPCWKLHTSPLCT
INTKEGSNICLRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTLPSLEVNLNCVDIFN
PKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNKNRGIKTFESNGCDYVSNKGVDTVSVG

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NTLYYVNQEGKSLYVKGEPINFYDPLVFPSEFDASISQVNEKINQSLAFIRKSDELLGGSM

EVLITVPSALAVAKIAHALVEERLAACVNIVPGLTSIYREEGSVVDHELLLKVITTDAPKL

KERVKELHPYEVPEIVALPIAEGNREYLDWLRENTG

DS-Cav1-foldon-T33-15B

(SEQ ID NO: 71)

(MELLILKANVIATILTAVTFCFASS) QNITEEFYQSTCSAVSKYLSALRTGWYTSVITIELSN

IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT

LSKKRKRRFLGFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL

DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL

SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCT

INTKEGSNICLRTDRGWYCDNAGSVFFFPAETCKVQSNRVCFTMNSLTLPEVNLNCNVDIFN

PKYDCKIMTSKTDVSSSVITSLGAIIVSCYGKTKCTASNKRGIIKTFSNGCDYVSNKGVDTSVG

NTLYYVNQEGKSLYVKGEPINFYDPLVFPSEFDASISQVNEKINQSLAFIRKSDELLGYIPE

APRDGQAYVRKDGEWLLSTFLGGSMVRGIRGAITVNSDTPTSIIATILLLEKMLEANGIQSYE

ELAAVIFTVTEDLTSAPPAEARQIGMHRVPLLSAREVPVPGSLPRVIRVLALWNTDTPQDRVRH

VYLSEAVRLRPDLESAQ

DS-Cav1-T33-15B

(SEQ ID NO: 72)

(MELLILKANVIATILTAVTFCFASS) QNITEEFYQSTCSAVSKYLSALRTGWYTSVITIELSN

IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT

LSKKRKRRFLGFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL

DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL

SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCT

INTKEGSNICLRTDRGWYCDNAGSVFFFPAETCKVQSNRVCFTMNSLTLPEVNLNCNVDIFN

PKYDCKIMTSKTDVSSSVITSLGAIIVSCYGKTKCTASNKRGIIKTFSNGCDYVSNKGVDTSVG

NTLYYVNQEGKSLYVKGEPINFYDPLVFPSEFDASISQVNEKINQSLAFIRKSDELLGGSMV

RGIRGAITVNSDTPTSIIATILLLEKMLEANGIQSYEELAAVIFTVTEDLTSAPPAEARQIGM

HRVPLLSAREVPVPGSLPRVIRVLALWNTDTPQDRVRHVYLSEAVRLRPDLESAQ

DS-Cav1-foldon-I53-50A

(SEQ ID NO: 73)

(MELLILKANAITTILTAVTFCFASS) QNITEEFYQSTCSAVSKYLSALRTGWYTSVITIELSN

IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT

LSKKRKRRFLGFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL

DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL

SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCT

INTKEGSNICLRTDRGWYCDNAGSVFFFPAETCKVQSNRVCFTMNSLTLPEVNLNCNVDIFN

PKYDCKIMTSKTDVSSSVITSLGAIIVSCYGKTKCTASNKRGIIKTFSNGCDYVSNKGVDTSVG

NTLYYVNQEGKSLYVKGEPINFYDPLVFPSEFDASISQVNEKINQSLAFIRGYIPEAPRDQ

AYVRKDGEWLLSTFLGSGSBBBBBBGGSGSGSEKAAKAEEARKMEELFKHKIVAVLRA

SVEEAIKEAVAVFAGGVHLIEITFTVADTVIKALSVLKKEKGAIIGAGTVTSVEQCRKAVESGA

EFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHТИЛКЛФГЕВВГРQFVKAMKGPFP

NVKFVPTGGVNLNVCEWFKAGVLAVGVGSALVKGT PDEVREKAFVEKIRGCTE

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DS-Cav1-I53-50A

(SEQ ID NO: 74)

(MELLILKANVIATILTAVTFCFASS) QNITEEFYQSTCSAVSKGYLESALRTGWTTSVITIELSN
 IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT
 LSKKRKRRLFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL
 DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL
 SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIKEEVLAYVVLPLYGVIDTPCWLHTSPLCT
 INTKEGSNICLRTDRGWYCDNAGSVSFPPQAETCKVQSNRVCDFTMNSLTPSEVNLNCNDIFN
 PKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVG
 NTLYYVNQEGKSLYVKGEPINFYDPLVFPSPDEFADASISQVNEKINQSLAFIRGSGGSGSEKA
 AKAEEAARKMEELFKKHKIVAVLRANSVEEAI EKAVAVFAGGVHLIEITFTV ррДАТВИКАСВЛ
 KEKGAIIGAGTVTSVEQCRKAVESGAЕFIVSPHLDEEISQFCKEKGVFYMPGMPTTELVKAMKL
 GHTILKLFPGЕVVGPQFVKAMGPFPNVKFVPTGGVNLDNCEWFKAGVLAVGVGSALVKGTPDE
 VREKAKAFVEKIRGCTE

DS-Cav1-I32-28A

(SEQ ID NO: 75)

(MELLILKANAIITILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLESALRTGWTTSVITIELSN
 IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT
 LSKKRKRRLFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL
 DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL
 SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIKEEVLAYVVLPLYGVIDTPCWLHTSPLCT
 INTKEGSNICLRTDRGWYCDNAGSVSFPPQAETCKVQSNRVCDFTMNSLTPSEVNLNCNDIFN
 PKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVG
 NTLYYVNQEGKSLYVKGEPINFYDPLVFPSPDEFADASISQVNEKINQSLAFIRKSDELLGGSGG
 SGSDARIAAIGDVDELNSQIGVLLAEPLPDDVRAALSAIQHDLFDLGGELCIPGHAAITEDHLL
 RLALWLVHYNGQLPPEEFILPGGARGAALAHVCRTVCRRERSIKALGASEPLNIAPAYVNLL
 SDLLFVLARVLNRAAGGADVLWDRTRAH

DS-Cav1-8GS-He1Ext-I53-50A (F10)

(SEQ ID NO: 76)

(MELLILKANAIITILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLESALRTGWTTSVITIELSN
 IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT
 LSKKRKRRLFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL
 DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL
 SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIKEEVLAYVVLPLYGVIDTPCWLHTSPLCT
 INTKEGSNICLRTDRGWYCDNAGSVSFPPQAETCKVQSNRVCDFTMNSLTPSEVNLNCNDIFN
 PKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASNKNRGIIKTFSNGCDYVSNKGVDTVSVG
 NTLYYVNQEGKSLYVKGEPINFYDPLVFPSPDEFADASISQVNEKINQSLAFIRGSGGSGSEKA
 AKAEEAARKMEELFKKHKIVAVLRANSVEEAI EKAVAVFAGGVHLIEITFTV ррДАТВИКАСВЛ
 KEKGAIIGAGTVTSVEQCRKAVESGAЕFIVSPHLDEEISQFCKEKGVFYMPGMPTTELVKAMKL
 GHTILKLFPGЕVVGPQFVKAMGPFPNVKFVPTGGVNLDNCEWFKAGVLAVGVGSALVKGTPDE
 VREKAKAFVEKIRGCTE

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DS-Cav1-foldon-15GS-He1Ext-I53-50A (F14) (SEQ ID NO: 77)
 (MELLILKANAITTILTAVTFCAFASQ QNITEEFYQSTCSAVSKGYSALRTGWYTSVITIELSN

IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT
 LSKKRKRRFLGFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL
 DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNRLLETREFSVNAGVTPVSTYMLTNSELL
 SLINDMPITNDQKQLMSNNVQIVRQQSYSIMCIKEEVLYAVVQLPLYGVIDTPCWKLHTSPLCT
 INTKEGSNICLRTDRGWYCDNAGSVFFPQAETCKVQSNRVFCDTMNSLTPSEVNLNCNDIFN
 PKYDCKIMTSKTDVSSSVITSGLAIVSCYGKTKCTASNKRGIICKTSNGCDYVSNKGVDTVSVG
 NTLYYVNVQEGKSLYVKGEPPIINFYDPLVFPSEFDASISQVNEKINQSLAFIRGYIPEAPRDQ
 AYVRKDGEWVLLSTFLGGSGSGSGSGSGEKAAKAEEAARKMEELFKKKIVAVLRANSVEEA
 IEKAVAVFAGGVHLIEITFTVPADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVS
 PHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFV
 PTGGVNLDNVCEWFKA GVLAVGVGSALVKGT PDEVREKAKAFVEKIRGCTE

HMPV F wt_Can97-83 strain-I53-50A (SEQ ID NO: 78)
 (MSWKVVIIFSLLITPQHG) LKESYLEESCSTITEGYLSVRTGWIINVFTLEVDVENCSDG

PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRFVLGAIALGVATAAAVTAGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSNDAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
 LIGVYGSVIIYMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWCQNAGSTVYPNE
 KDCETRGDHVFCDTAACINVAEKSKECNINISTTNYPCKVSTGRHPISMVALSPLAGLVACYKGV
 SCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGFIIVIIIAVLGSSMILVSIFIKKTK
 KPTGAPP else LSGVTNNGFIPHSGSGSHHHHHHHGGSGSGSEKAAKAEEAARKMEELFKKKIV
 VLRANSVEEAIEKAVAVFAGGVHLIEITFTVPADTVIKALSVLKEKGAIIGAGTVTSVEQCRKA
 VESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAM
 KGPFPNVKFVPTGGVNLDNVCEWFKA GVLAVGVGSALVKGT PDEVREKAKAFVEKIRGCTE

HMPV F A113C_A339C_T160F_I177L-I53-50A (SEQ ID NO: 79)
 (MSWKVVIIFSLLITPQHG) LKESYLEESCSTITEGYLSVRTGWIINVFTLEVDVENCSDG

PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRFVLGAIALGVCTAAVTAGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSNDAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
 LIGVYGSVIIYMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWCQNAGSTVYPNE
 KDCETRGDHVFCDTAACINVAEKSKECNINISTTNYPCKVSTGRHPISMVALSPLAGLVACYKGV
 SCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGGSGSHHHHHHHGGSGSGSEKAAKAEE
 AARKMEELFKKKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPADTVIKALSVLKEKGAI
 IIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTIL
 KLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKA GVLAVGVGSALVKGT PDEVREKA
 KAFVEKIRGCTE

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HMPV F A113C_A339C_T160F_I177L_A120C, Q426C mutations-I53-50A
 (SEQ ID NO: 80)
 (MSWKVVIIFSLITPQHG) LKESYLEESCSTITEGILSVLRTGWIINVFTLEVDVENLICSDG

PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRFLGAIALGVCTAAAVTCGVAIAKTI
 RLESEVTAKNALKTTNEAVSTLGNNGVRVLAFAVRELKDFVSKNLTRALNKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSDNAGITPAISLDMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
 LIGVYGSVSIYMVQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWYCQNAGSTVYPNE
 KDCETRGDHVFCDTACGINVABQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACYKGV
 SCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYCLS KVEGEQHVIKGRPVSSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGGSGSHHHHHHHGGSGGSEKAAKAEE
 AARKMEELFKHKIVAVLRANSVEEAIKAVAVFAGGVHLIEITFTVPDADTVIKALSVLK
 IIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGH
 KLFPGEVVGPQFVKAMGPFPNVKFVPTGGVNLDNVCEWFKAGVLAGVGVSALVKGT
 KAFVEKIRGCTE

sc-DS2-I53-50A
 (SEQ ID NO: 81)
 (MELLILKANAITTILTAVTFCAFASG) QNITEEFYQSTCSAVSKGYLSALRTGWT
 VITIELSN

IKENKCNGTDAKVKLICKQELDKYKNAVTELQLLMQSTPATGSGSCI
 ASGVAVCKVLHLEG
 VNK
 KSALLSTNKAVVSLNSNGVS
 VLT
 FKVL
 DLKNYIDKQLL
 PI
 LNQ
 SC
 SIS
 NI
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 VIE
 FQQ
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tc-DS2-I53-50A
 (SEQ ID NO: 82)
 (MELLILKANAITTILTAVTFCAFASG) QNITEEFYQSTCSAVSKGYLSALRTGWT
 VITIELSN

IKENKCNGTDAKVKLICKQELDKYKNAVTELQLLMQSTPATNNR
 ARREL
 PRFM
 NYTL
 NNAK
 KTN
 VT
 LSKKR
 RR
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 Q
 F
 V
 K
 A
 M
 G
 P
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 V
 K
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DS-Cav1-12GS-HeIExt -I53-50A (F11)

(SEQ ID NO: 83)

(MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLSALRTGWYTsvitielsn
 IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT
 LSKKRKRFLGFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL
 DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL
 SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCT
 INTKEGSNICLRTDRGWYCDNAGSVSFPPQAETCKVQSNRVCDFTMNSLTPSEVNLNCNVDIFN
 PKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASNKRGIIKTFSNGCDYVSNKGVDTVSVG
 NTLYYVNQEGKSLYVKGEP I INFYDPLVFPSPDEF DASISQVNEKINQSLAFIRGSGGSGGG
 SEKAAKAEEAARKMEELFKHKIVAVL RANSVEEAI EKAVAVFAGGVHLIE1IFTVPDADTVKA
 LSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPELVK
 AMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAvgVGSA
 TPDEVREKAKAFVEKIRGCTE

DS-Cav1-16GS-HeIExt -I53-50A (F12)

(SEQ ID NO: 84)

(MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLSALRTGWYTsvitielsn
 IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT
 LSKKRKRFLGFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL
 DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL
 SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCT
 INTKEGSNICLRTDRGWYCDNAGSVSFPPQAETCKVQSNRVCDFTMNSLTPSEVNLNCNVDIFN
 PKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASNKRGIIKTFSNGCDYVSNKGVDTVSVG
 NTLYYVNQEGKSLYVKGEP I INFYDPLVFPSPDEF DASISQVNEKINQSLAFIRGSGGSGGG
 SGSGGEAKAEEAARKMEELFKHKIVAVL RANSVEEAI EKAVAVFAGGVHLIE1IFTVPDADTVKA
 VIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPELVK
 ELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKAGVLAvgVGSA
 LVKGTPDEVREKAKAFVEKIRGCTE

DS-Cav1-foldon-10GS-HeIExt -I53-50A (F13)

(SEQ ID NO: 85)

(MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLSALRTGWYTsvitielsn
 IKENKCNGTDAVKLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT
 LSKKRKRFLGFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL
 DLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELL
 SLINDMPITNDQKKLMSNNVQIVRQQSYSIMCIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCT
 INTKEGSNICLRTDRGWYCDNAGSVSFPPQAETCKVQSNRVCDFTMNSLTPSEVNLNCNVDIFN
 PKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASNKRGIIKTFSNGCDYVSNKGVDTVSVG
 NTLYYVNQEGKSLYVKGEP I INFYDPLVFPSPDEF DASISQVNEKINQSLAFIRGYIPEAPRDQ
 AYVRKDGEWLLSTFLGSGGSGSGEKAAKAEEAARKMEELFKHKIVAVL RANSVEEAI EKAV
 AVFAGGVHLIE1IFTVPDADTVKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDE
 EISQFCKEKGVFYMPGVMTPELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGV
 NLDNVCEWFKAGVLAvgVGSA
 LVKGTPDEVREKAKAFVEKIRGCTE

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DS-Cav1-foldon-20GS-He1Ext-I53-50A (F15) (SEQ ID NO: 86)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLESALRTGWTTSVITIELSN

IKENKCNGTDAKVLIKQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMNYTLNNAKTNVT
 LSKKRKRRFLGFLGVGSIAISGVAVCKVLHLEGEVNKIKSALLSTNKAVVSLNSNGVSVLTFKVL
 DLKNYIDKQLLPILNQSCSISNIETVIEFQQKNRLLETREFSVNAGVTPVSTYMLTNSELL
 SLINDMPITNDQKKLMSNNVQIVRQSYSIMCIKEEVLYAVVQLPLYGVIDTPCWKLHTSPLCT
 INTKEGSNICLRTDRGWYCDNAGSVSFFPQAETCKVQSNRVFCDTMNSLTPSEVNLNCNVDIFN
 PKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASNKRGIIKTFSNGCDYVSNKGVDTVSVG
 NTLYYVNVQEGKSLYVKGEPINFYDPLVFPSEFDASI SQVNEKINQSLAFIRGYIPEAPRDQ
 AYVRKDGEWVLLSTFLGGSGSGSGSGSGSGSEKAAKAEEAARKMEELFKKHKIVAVLRA
 SVEEAIEKAVAVFAGGVHLIEITFTVADTVIKALSVLKKEKGAIIGAGTVTSVEQCRKAVESGA
 EFIGSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHТИLKFPGEVVGPQFVKAMKGPF
 NVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTDEVREKAKAFVEKIRGCTE

sc9-10 DS-Cav1 A149C Y458C-foldon-I53-50A embodiment (SEQ ID NO: 87)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLESALRTGWTTSVITIELSN

IKENKCNGTDAKVLIKQELDKYKNAVTELQLLMQSTPATGSGSAICSGVAVCKVLHLEGEVNKI
 KSALLSTNKAVVSLNSNGVSVLTKVLDLKNYIDKQLLPILNQSCSISNIETVIEFQQKNRLL
 ITREFSVNAGVTPVSTYMLINSELLSLINDMPITNDQKKLMSNNVQIVRQSYSIMCIKEEV
 AVVQLPLYGVIDTPCWKLHTSPLCTINTKEGSNICLRTDRGWYCDNAGSVSFFPQAETCKVQS
 NRVFCDTMNSRTLPSEVNLNCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASN
 NRGIIKTFSNGCDYVSNKGVDTSVGNTLYCVNKQEGKSLYVKGEPINFYDPLVFPSEFDASI
 SQVNEKINQSLAFIR (KSDELL) GYIPEAPRDQAVRKDGEWVLLSTFLGGSGHHHHHHHHGG
 GGSGSEKAAKAEEAARKMEELFKKHKIVAVLRA NSVEEAIEKAVAVFAGGVHLIEITFTVAD
 VIKALSVLKKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMT
 ELVKAMKLGHТИLKFPGEVVGPQFVKAMKGPFPNVKVPTGGVNLDNVCEWFKAGVLAVGVSA
 LVKGTPDEVREKAKAFVEKIRGCTE

sc9-10 DS-Cav1 A149C Y458C-I53-50A - F10 embodiment (SEQ ID NO: 88)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKGYLESALRTGWTTSVITIELSN

IKENKCNGTDAKVLIKQELDKYKNAVTELQLLMQSTPATGSGSAICSGVAVCKVLHLEGEVNKI
 KSALLSTNKAVVSLNSNGVSVLTKVLDLKNYIDKQLLPILNQSCSISNIETVIEFQQKNRLL
 ITREFSVNAGVTPVSTYMLINSELLSLINDMPITNDQKKLMSNNVQIVRQSYSIMCIKEEV
 AVVQLPLYGVIDTPCWKLHTSPLCTINTKEGSNICLRTDRGWYCDNAGSVSFFPQAETCKVQS
 NRVFCDTMNSRTLPSEVNLNCNVDIFNPKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASN
 NRGIIKTFSNGCDYVSNKGVDTSVGNTLYCVNKQEGKSLYVKGEPINFYDPLVFPSEFDASI
 SQVNEKINQSLAFIR (KSDELL) GGGSGSGEKAAKAEEAARKMEELFKKHKIVAVLRA NSVEEA
 IEKAVAVFAGGVHLIEITFTVADTVIKALSVLKKEKGAIIGAGTVTSVEQCRKAVESGAEFIV
 PHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHТИLKFPGEVVGPQFVKAMKGPFPNVKV
 PTGGVNLDNVCEWFKAGVLAVGVGSALVKGTDEVREKAKAFVEKIRGCTE

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sc9-10 DS-Cav1 A149C Y458C S46G K465Q S215P E92D-foldon-I53-50A
embodiment (SEQ ID NO: 89)

I KENKCNGTDAKVLIKQELDKYKNAVTDLQLLMQSTPATGSGSAICSGVAVCKVLHLEGEVNKI
KSALLSTNKAVVSLNSNGVSULTFVKVLNLKNYIDKQLLPILNQSCSIPNIETVIEFQQKNRLL
ITREFSVNAGVTPVSTYMLINSELLSLINDMPI TNDQKKLMSNNVQIVRQQSYSIMCIKEEVL
AYVVQLPLYGVIDDTCPCWKLHTSPCLTTNTKEGSNICLRTDRGWYCDNAGSVSFFPQAETCKVQS
NRVFCDTMNSRTLPSVENLCNVDFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCTASNK
NRGIIKTFNSGCDYVSNKGDTVSVGNTLYCVNKQEGQSLYVKGEPI INFYDPLVFPSEDFDASI
SQVNEKINQSLAFIR (KSDELL) GYIPEAPRDQAYVRKDGEWLLSTFLGSGSHHHHHHHGGS
GGSGSEKAAKAAAARKMEELFKKKHVIAVLRANSVEEAIKAVAVFAGGVHLIEL1FTVPADT
VIKALSVLKKGAIIGAGTVSVEQCRKAVEGSAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPT
ELVKAMKLGHТИKLFPGEVVGPKVKAMKGPFPNVKFVPTGGVNLDNVCEWPKAGVLAVGVGSA
LVKGTPDEVREKAKFVEKIRGCTE

sc9-10 DS-Cav1 A149C Y458C S46G K465Q S215P E92D-I53-50A - F10
embodiment (SEQ ID NO: 90)
(MELLILKANAITTILTAVTFCAFASG) QNITEEFYQSTCSAVSKGYLGALRTGWYTSVITELSN

I KENKCNGTDAVKVLIKQELDKYNAVTDLQLLMQSTPATGSGSAICSGVAVCKVLHLEGEVNKI
KSALLSTNKAVVSLNSGVSLTFVKVLDLKNYIDKQLLPILNKQSCSIPNIEVIEFQQKNNRLL
ITREFSVNAGVTTPVSTYMLINSELLSLINDMPITNDQKQLMSNNVQIVRQQSYSIMCIKEEVL
AYVVQLPLYGVIDDTPCWLHTSPLCTTNTKEGSNICLRTDRGWYCDNAGGSVSFFPQAETCKVQS
NRVFCDTMNSRTLPSLEVNLNVDFNPKYDCKIMTSKTDVSSSVITSLGAI VSCYGKTKCTASNK
NRGIIKTFNSNGCDYVSNKGDTVSVGNLYCVNKQEGQSLYVKGEPIINFYDPLVFPSSDEFDASI
SQVNEKINQSLAFIR (KSELL) GSGGSGSGEKAAKAEEAARKMEELFKKHKIVAVLTRANSVEEA
IEKAVAVFAGGVHLIEITFTVADTVIKALSVLKEKGAIIGAGTVTSVQEQRKAVEGSAEFIVS
PHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHТИKLFPGEVVGPFQFVKAMKGPFPNVKFV
PTGGVNLDNVCEWFKAQGLAVGVGSALVKGTPDEVREKAKAFVEKIRGCTE

SC-DM (N67I, S215P) - foldon-I53-50A embodiment
(SEQ ID NO: 91)
(MELLILKANAITTILTAVTFCCFASG) QNITEEFYQSTCSAVSKGYLALSALRTGWYTSVITELSN

IKKIKCNGTDAKIKLIKQELDKYKNAVTELQLLMQSTPATNNQARGSGSGRSGLFGFLVGVSIAIS
GVAVSKVLHLEGEVNKIKSALLSTNKAVVSLNSNGSVLTSKVLDLKNYIDKQLLPIVNKQSCSIP
NIETVIEFQQKNRNLLEITREFSVNAGVTPVSTYMLNSELLSLINDMPITNDQKKLMSNNVQI
VRQQSYSIMSIIKEEVLAYVQLPLYGVIDTPCWKLHTSPCLTTNTKEGSNICLRTDRGWCND
AGSVSFPPQAETCKVQSNRVCDFTMNSLTLPSNEVLCNVDIFNPKYDCKIMTSKTDVSSSVITSL
GAIVSCYGKTCTASNKNRGIIKTFNSNGCDYVSNKGVDTSVGNTLYVNQEGKSILYVKGEPII
NFYDPLVFPSPDEFDASIISQVNEKINQSLAFIR (KSEDELL) GYIPEAPRDGQAYVRKDGEWLLST
FLGSGSHHHHHHHGGSGSGSEKAAKAEEAARKMEELFKKHKIVAVLTRANSVEEAIEKAVAVFA
GGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEIFVSPHLDEEISQ
FCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDN
VCEWFKAGVLAVGVGVSALVKGTPDEVREKAKAFVKEIRGCTE

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SC-DM (N67I, S215P) - I53-50A - F10 embodiment
 (SEQ ID NO: 92)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKYLSALRTGWYTSVITIELSN

IKKIKCNGTDAKIKLIKQELDKYKNAVTELQLLMQSTPATNNQARGSGSGRSLGFLGVGSAIAS
 GVAWSKVLHLEGEVNIKSALLSTNKAVVSLNSNGSVLTSKVLSDLKNYIDKQLLPVNQSCSIP
 NIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELLSINDMPITNDQKKLMSNNVQI
 VRQQSYSIMSIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCTNTKEGSNICLRTDRGWYCDN
 AGSVSFFFPAETCKVQSNRVCFTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITS
 GAIIVSCYGKTCTASNKNRGIKTFNSNGCDYVSNKGVDTSVGNTLYYVNQEGKSLYVKGEPII
 NFYDPLVPPSDQFDASIISQVNEKINQSLAFIR (KSDELL) GSGGSGSGEAKAAEARKMEELF
 KKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTS
 VEQCRKAVESGAEFIVSPHLDEEISQFCCKEKGVFYMPGVMTPTELVKAMKLGHТИKLFPGEVVG
 PQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKA GVLA VGVGSALVKGTPDEVREKAKAFVEKIRG

CTE

SC-TM (N67I, S215P, and E487Q) - foldon-I53-50A embodiment
 (SEQ ID NO: 93)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKYLSALRTGWYTSVITIELSN

IKKIKCNGTDAKIKLIKQELDKYKNAVTELQLLMQSTPATNNQARGSGSGRSLGFLGVGSAIAS
 GVAWSKVLHLEGEVNIKSALLSTNKAVVSLNSNGSVLTSKVLSDLKNYIDKQLLPVNQSCSIP
 NIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELLSINDMPITNDQKKLMSNNVQI
 VRQQSYSIMSIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCTNTKEGSNICLRTDRGWYCDN
 AGSVSFFFPAETCKVQSNRVCFTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITS
 GAIIVSCYGKTCTASNKNRGIKTFNSNGCDYVSNKGVDTSVGNTLYYVNQEGKSLYVKGEPII
 NFYDPLVPPSDQFDASIISQVNEKINQSLAFIR (KSDELL) GYIPEAPRDGQAYVRKDGEWVLST
 FLGSGSHHHHHHHGGSGSGEAKAAEARKMEELFKHKIVAVLRANSVEEAIEKAVAVFA
 GGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQ
 FCCKEKGVFYMPGVMTPTELVKAMKLGHТИKLFPGEVVGPFQVKAMKGPFPNVKFVPTGGVNLDN
 VCEWFKA GVLA VGVGSALVKGTPDEVREKAKAFVEKIRGCTE

SC-TM (N67I, S215P, and E487Q) - I53-50A - F10 embodiment
 (SEQ ID NO: 94)
 (MELLILKANAITTILTAVTFCFASG) QNITEEFYQSTCSAVSKYLSALRTGWYTSVITIELSN

IKKIKCNGTDAKIKLIKQELDKYKNAVTELQLLMQSTPATNNQARGSGSGRSLGFLGVGSAIAS
 GVAWSKVLHLEGEVNIKSALLSTNKAVVSLNSNGSVLTSKVLSDLKNYIDKQLLPVNQSCSIP
 NIETVIEFQQKNNRLLEITREFSVNAGVTPVSTYMLTNSELLSINDMPITNDQKKLMSNNVQI
 VRQQSYSIMSIIKEEVLAYVQLPLYGVIDTPCWKLHTSPLCTNTKEGSNICLRTDRGWYCDN
 AGSVSFFFPAETCKVQSNRVCFTMNSLTLPSEVNLCNVDIFNPKYDCKIMTSKTDVSSSVITS
 GAIIVSCYGKTCTASNKNRGIKTFNSNGCDYVSNKGVDTSVGNTLYYVNQEGKSLYVKGEPII
 NFYDPLVPPSDQFDASIISQVNEKINQSLAFIR (KSDELL) GSGGSGSGEAKAAEARKMEELF
 KKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKEKGAIIGAGTVTS
 VEQCRKAVESGAEFIVSPHLDEEISQFCCKEKGVFYMPGVMTPTELVKAMKLGHТИKLFPGEVVG
 PQFVKAMKGPFPNVKFVPTGGVNLDNVCEWFKA GVLA VGVGSALVKGTPDEVREKAKAFVEKIRG

CTE

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HMPV-F with A113C, A339C, T160F, I177L - foldon-I53-50A embodiment
 (SEQ ID NO: 95)
 (MSWKVVIIFSLLITPQHG) LKESYLEESCSTITEGYSVLRTGWYTNVFTLEVGDVENLTCSDG

PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRVLGAIALGVCTAAAVTAGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLAFAVRELKDFVSKNLTRALNKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSNDAGITPAISLDMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
 LIGVYGSVIVMQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWYCQNAGSTVYPNE
 KDCETRGDHVFCDTACGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPGLALVACYKG
 SCSIGSNRVGIKQLNKGSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGGYIPEAPRDGQAYVRKDGEWVLLSTFLG
 SGSHHHHHHHGGSGSGSEKAAKAEEARKMEELFKKHIVAVLTRANSVEEAIEKAVAVFAGGV
 HLIEITFTVPDADTVIKALSVLKKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCK
 EKGVFYMPGVMTPTELVKAMKLGHТИKLFPGEVVGQFVKAMGPFPNVKFVPTGGVNLDNVCE
 WFKAGVLAvgvgsalvkgtpdevrekafvekrgcte

HMPV-F with A113C, A339C, T160F, I177L-I53-50A F10 embodiment
 (SEQ ID NO: 96)
 (MSWKVVIIFSLLITPQHG) LKESYLEESCSTITEGYSVLRTGWYTNVFTLEVGDVENLTCSDG

PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRVLGAIALGVCTAAAVTAGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLAFAVRELKDFVSKNLTRALNKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSNDAGITPAISLDMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
 LIGVYGSVIVMQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWYCQNAGSTVYPNE
 KDCETRGDHVFCDTACGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPGLALVACYKG
 SCSIGSNRVGIKQLNKGSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGGSGSGSEKAAKAEEARKMEELFKH
 KIVAVLTRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSVLKKEKGAIIGAGTVTSVEQ
 CRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHТИKLFPGEVVGQF
 VKAMGPFPNVKFVPTGGVNLDNVCEWFKAGVLAvgvgsalvkgtpdevrekafvekrgcte

HMPV-F with A113C, A120C, A339C, T160F, I177L, and Q426C - foldon-I53-50A embodiment
 (SEQ ID NO: 97)
 (MSWKVVIIFSLLITPQHG) LKESYLEESCSTITEGYSVLRTGWYINVFTLEVGDVENLICSDG

PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRVLGAIALGVCTAAAVTCGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLAFAVRELKDFVSKNLTRALNKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSNDAGITPAISLDMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFGI
 LIGVYGSVIVMQLPIFGVIDTPCWIVKAAPSCSGKKGNYACLLREDQGWYCQNAGSTVYPNE
 KDCETRGDHVFCDTACGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPGLALVACYKG
 SCSIGSNRVGIKQLNKGSYITNQDADTVTIDNTVYCLS KVEGEQHVIKGRPVSSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGGYIPEAPRDGQAYVRKDGEWVLLSTFLG
 SGSHHHHHHHGGSGSGSEKAAKAEEARKMEELFKKHIVAVLTRANSVEEAIEKAVAVFAGGV
 HLIEITFTVPDADTVIKALSVLKKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCK
 EKGVFYMPGVMTPTELVKAMKLGHТИKLFPGEVVGQFVKAMGPFPNVKFVPTGGVNLDNVCE
 WFKAGVLAvgvgsalvkgtpdevrekafvekrgcte

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HMPV-F with A113C, A120C, A339C, T160F, I177L, and Q426C - F10 embodiment
 (SEQ ID NO: 98)

(MSWKVIIIFSLLITPQHG) LKESYLEESCSTITEGYLSVLRTGWIINVFTLEVDVENLICSDG

PSLIKTELDLTKSALRELKTVSADQLAREEQIENPRQSRFVLGAIALGVATAAVTCVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLAFAVRELKDFVSKNLTRALNKNKCDIDDLKMAVSF
 SQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFI
 LIGVYGSIVIYMVQLPIFGVIDTPCWIVKAAPSCSGKGNYACLLREDQGWCQNAGSTVYPNE
 KDCETRGDHVFCDTACGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLAGVACYKGV
 SCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYCLSKEVEQHVIKGRPVSSSFDPIKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGGSGSGSGEKAAKAEEARKMEELFKKH
 KIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVADTVIKALSVLKKEKGAIIGAGTVTSVEQ
 CRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHТИLKFPGEVVGPQF
 VKAMKGPPNPKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTDEVREKAKAFVEKIRGCTE

HMPV-F 115-BV (A185P) -foldon-I53-50A embodiment

(SEQ ID NO: 99)

(MSWKVIIIFSLLITPQHG) LKESYLEESCSTITEGYLSVLRTGWIINVFTLEVDVENLICADG

PSLIKTELDLIKSALRELRIVSADQLAREEQIENPRRRRFVLGAIALGVATAAVTAGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSF
 SQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFI
 LIGVYGSIVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWCQNAGSTVYPNE
 KDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLAGVACYKGV
 SCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQOLSKEVEQHVIKGRPVSSSFDPVKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGYIPEAPRDQAYVRKDGEWVLLSTFLGS
 GSHHHHHHHGGSGSGSEKAAKAEEARKMEELFKKHIVAVLRANSVEEAIEKAVAVFAGGVH
 LIEITFTVADTVIKALSVLKKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKE
 KGVFYMPGVMTPTELVKAMKLGHТИLKFPGEVVGPQFVKAMKGPPNPKFVPTGGVNLDNVCEW
 FKAGVLAVGVGSALVKGTDEVREKAKAFVEKIRGCTE

HMPV-F 115-BV (A185P) -I53-50A - F10 embodiment

(SEQ ID NO: 100)

(MSWKVIIIFSLLITPQHG) LKESYLEESCSTITEGYLSVLRTGWIINVFTLEVDVENLICADG

PSLIKTELDLIKSALRELRIVSADQLAREEQIENPRRRRFVLGAIALGVATAAVTAGVIAKTI
 RLESEVTAIKNALKTNEAVSTLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAVSF
 SQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMPTSAGQIKLMLENRAMVRRKGFI
 LIGVYGSIVIYMVQLPIFGVIDTPCWIVKAAPSCSEKKGNYACLLREDQGWCQNAGSTVYPNE
 KDCETRGDHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLAGVACYKGV
 SCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQOLSKEVEQHVIKGRPVSSSFDPVKFPE
 DQFNVALDQVFENIENSQALVDQSNRILSSAEKGNTGGSGSGEKAAKAEEARKMEELFKKH
 IVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVADTVIKALSVLKKEKGAIIGAGTVTSVEQC
 CRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHТИLKFPGEVVGPQFV
 VKAMKGPPNPKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTDEVREKAKAFVEKIRGCTE

Second Assemblies

[0128] The nanostructures of the invention may comprise multiple copies of a trimeric first assembly and multiple copies of a second assembly. The second assembly comprises a protein-protein interface that induces multiple copies of the second polypeptide to self-associate to form the second assemblies. Multiple oligomeric states of the second assembly may be compatible with nanostructure formation, including dimeric (two copies), trimeric (three copies), tetrameric (four copies), pentameric (five copies), hexameric (six copies), or higher oligomeric states. Each copy of the second assembly further comprises a surface-exposed interface that interacts with a complementary surface-exposed interface on a trimeric assembly domain. As described in King et al., Bale et al., and patent publications WO2014124301 A1 and US20160122392 A1, the complementary interface between the trimeric assembly domain and second assembly domain drives the assembly of multiple copies of the trimeric assembly domain and second assembly domain to a target nanostructure. In various specific embodiments:

[0129] (a) when each first polypeptide is DS-Cav1-foldon-T33-31A (SEQ ID NO:69) or DS-Cav1-T33-31A (SEQ ID NO:70), each second polypeptide is T33-31B (SEQ ID NO:44);

[0130] (b) when each first polypeptide is DS-Cav1-foldon-T33-15B (SEQ ID NO:71) or DS-Cav1-T33-15B (SEQ ID NO:72), each second polypeptide is T33-15A (SEQ ID NO:45);

[0131] (c) when each first polypeptide is DS-Cav1-foldon-I53-50A (SEQ ID NO:73) or DS-Cav1-I53-50A (SEQ ID NO:74), each second polypeptide is I53-50B (SEQ ID NO:8), I53-50B.1 (SEQ ID NO:32), I53-50B.1NegT2 (SEQ ID NO:33), or I53-50B.4PosT1 (SEQ ID NO:34);

[0132] (d) when each first polypeptide is DS-Cav1-I32-28A (SEQ ID NO:75), each second polypeptide is I32-28B.

Assembly of Full Valency Nanostructures by In Vitro Assembly of Two Components

[0133] In some embodiments, each trimeric first assembly of the nanostructure bears an identical F protein as a genetic fusion; these nanostructures display the F protein at full (100%) valency. Such nanostructures are produced from purified first polypeptides and second polypeptides in a process called in vitro assembly. Purified trimeric first polypeptides comprising an F protein, are mixed with appropriate second polypeptides in an approximately 1:1 molar ratio in aqueous conditions (see FIG. 1). The second assembly interacts with the trimeric first assembly in order to drive assembly of the target nanostructure. Successful assembly of the target nanostructure can be confirmed by analyzing the in vitro assembly reaction by common biochemical or biophysical methods used to assess the physical size of proteins or protein assemblies, including but not limited to size exclusion chromatography, native (non-denaturing) gel electrophoresis, dynamic light scattering, multi-angle light scattering, analytical ultracentrifugation, negative stain electron microscopy, cryo-electron microscopy, or X-ray crystallography. If necessary, the assembled nanostructure can be purified from other species or molecules present in the in vitro assembly reaction using preparative techniques com-

monly used to isolate proteins by their physical size, including but not limited to size exclusion chromatography, preparative ultracentrifugation, tangential flow filtration, or preparative gel electrophoresis. The presence of the F protein in the nanostructure can be assessed by techniques commonly used to determine the identity of protein molecules in aqueous solutions, including but not limited to SDS-PAGE, mass spectrometry, protein sequencing, or amino acid analysis. The accessibility of the F protein on the exterior of the particle, as well as its conformation or antigenicity, can be assessed by techniques commonly used to detect the presence and conformation of an antigen, including but not limited to binding by monoclonal antibodies, conformation-specific monoclonal antibodies, or antisera specific to the antigen.

In Vitro Assembly of Partial Valency Nanostructures

[0134] In other embodiments, the nanostructures of the invention comprise one or more copies of trimeric first assemblies bearing F proteins as genetic fusions as well as one or more trimeric first assemblies that do not bear F proteins as genetic fusions; these nanostructures display the F proteins at partial valency. These partial valency nanostructures are produced by performing in vitro assembly with mixtures of first polypeptides in which the fraction of trimeric first assemblies bearing an F protein as a genetic fusion is equal to the desired valency of the antigen in the resulting nanostructure. The in vitro assembly reaction typically contains an approximately 1:1 molar ratio of total first polypeptides to total second polypeptides. By way of non-limiting example, performing an in vitro assembly reaction with a mixture of trimeric assemblies in which one half of the first polypeptides bear an F protein as a genetic fusion would yield an assembled nanostructure with an F protein valency of 50%. That is, 50% of the possible sites for F protein display on the nanostructure would be occupied. By way of non-limiting example, if the nanostructure is a 120-subunit assembly with icosahedral symmetry, the nanostructure comprises 20 total trimeric building blocks, and a 50% valency nanostructure displays 10 of the possible 20 F protein trimers. In this way, the ratio of F protein-bearing first polypeptides to first polypeptides lacking F proteins in an in vitro assembly reaction can be used to precisely tune the F protein valency of the resulting nanostructures. It will be understood by those of skill in the art that it is the average valency that can be tuned in this manner; the valency of individual nanostructures in the mixture will be a distribution centered around the average. Successful assembly of such partial valency nanostructures can be assessed using the techniques described above for evaluating full-valency nanostructures, and, if necessary, the partial valency nanostructures can be purified using the methods described for purifying full-valency nanostructures. The average valency of F protein-bearing first polypeptides in a given sample can be assessed by quantitative analysis using the techniques described above for evaluating the presence of F proteins in full-valency nanostructures.

In Vitro Assembly of Nanostructures Co-Displaying Multiple F Proteins

[0135] In other embodiments, the nanostructures of the invention comprise two or more distinct first polypeptides bearing different F proteins as genetic fusions; these nano-

structures co-display multiple different F proteins on the same nanostructure. These multi-antigen nanostructures are produced by performing in vitro assembly with mixtures of first polypeptides in which each first polypeptide bears one of two or more distinct F proteins as a genetic fusion. The fraction of each first polypeptide in the mixture determines the average valency of each F protein in the resulting nanostructures. The in vitro assembly reaction typically contains an approximately 1:1 molar ratio of total trimeric first polypeptides to total second polypeptides. The presence and average valency of each F protein-bearing first polypeptides in a given sample can be assessed by quantitative analysis using the techniques described above for evaluating the presence of F proteins in full-valency nanostructures.

[0136] In various embodiments, the nanostructures are between about 20 nanometers (nm) to about 40 nm in diameter, with interior lumens between about 15 nm to about 32 nm across and pore sizes in the protein shells between about 1 nm to about 14 nm in their longest dimensions.

[0137] In one embodiment, the nanostructure has icosahedral symmetry. In this embodiment, the nanostructure may comprise 60 copies of the first polypeptide and 60 copies of the second polypeptide. In one such embodiment, the number of identical first polypeptides in each first assembly is different than the number of identical second polypeptides in each second assembly. For example, in one embodiment, the nanostructure comprises twelve first assemblies and twenty second assemblies; in this embodiment, each first assembly may, for example, comprise five copies of the identical first polypeptide, and each second assembly may, for example, comprise three copies of the identical second polypeptide. In another embodiment, the nanostructure comprises twelve first assemblies and thirty second assemblies; in this embodiment, each first assembly may, for example, comprise five copies of the identical first polypeptide, and each second assembly may, for example, comprise two copies of the identical second polypeptide. In a further embodiment, the nanostructure comprises twenty first assemblies and thirty second assemblies; in this embodiment, each first assembly may, for example, comprise three copies of the identical first polypeptide, and each second assembly may, for example, comprise two copies of the identical second polypeptide. All of these embodiments are capable of forming synthetic nanomaterials with regular icosahedral symmetry. In various further embodiments, oligomeric states of the first and second polypeptides are as follows:

- [0138] I53-34A: trimer+I53-34B: pentamer;
- [0139] I53-40A: pentamer+I53-40B: trimer;
- [0140] I53-47A: trimer+I53-47B: pentamer;
- [0141] I53-50A: trimer+I53-50B: pentamer;
- [0142] I53-51A: trimer+I53-51B: pentamer;
- [0143] I32-06A: dimer+I32-06B: trimer;
- [0144] I32-19A: trimer+I32-19B: dimer;
- [0145] I32-28A: trimer+I32-28B: dimer;
- [0146] I52-03A: pentamer+I52-03B: dimer;
- [0147] I52-32A: dimer+I52-32B: pentamer; and
- [0148] I52-33A: pentamer+I52-33B: dimer

[0149] In another embodiment, the nanostructure of any embodiment or combination of embodiments of the invention has one or more of the following characteristics, each as demonstrated in the examples that follow:

- [0150] (a) binds prefusion F-specific antibodies including but not limited to monoclonal antibody D25;

[0151] (b) forms a symmetrical structure, including but not limited to an icosahedral structure;

[0152] (c) is stable at 50° C.; and/or

[0153] (d) is stable in 2.25M guanidine hydrochloride.

[0154] In another aspect, the present invention provides isolated nucleic acids encoding a fusion protein of the present invention. The isolated nucleic acid sequence may comprise RNA or DNA. As used herein, "isolated nucleic acids" are those that have been removed from their normal surrounding nucleic acid sequences in the genome or in cDNA sequences. Such isolated nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals. It will be apparent to those of skill in the art, based on the teachings herein, what nucleic acid sequences will encode the proteins of the invention.

[0155] In a further aspect, the present invention provides recombinant expression vectors comprising the isolated nucleic acid of any embodiment or combination of embodiments of the invention operatively linked to a suitable control sequence. "Recombinant expression vector" includes vectors that operatively link a nucleic acid coding region or gene to any control sequences capable of effecting expression of the gene product. "Control sequences" operably linked to the nucleic acid sequences of the invention are nucleic acid sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Thus, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors can be of any type known in the art, including but not limited to plasmid and viral-based expression vectors. The control sequence used to drive expression of the disclosed nucleic acid sequences in a mammalian system may be constitutive (driven by any of a variety of promoters, including but not limited to, CMV, SV40, RSV, actin, EF) or inducible (driven by any of a number of inducible promoters including, but not limited to, tetracycline, ecdysone, steroid-responsive). The construction of expression vectors for use in transfecting prokaryotic cells is also well known in the art, and thus can be accomplished via standard techniques. (See, for example, Sambrook, Fritsch, and Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989; Gene Transfer and Expression Protocols, pp. 109-128, ed. E. J. Murray, The Humana Press Inc., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX). The expression vector must be replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In a preferred embodiment, the expression vector comprises a plasmid. However, the invention is intended to include other expression vectors that serve equivalent functions, such as viral vectors.

[0156] In another aspect, the present invention provides host cells that have been transfected with the recombinant expression vectors disclosed herein, wherein the host cells

can be either prokaryotic or eukaryotic, such as mammalian cells. The cells can be transiently or stably transfected. Such transfection of expression vectors into prokaryotic and eukaryotic cells can be accomplished via any technique known in the art, including but not limited to standard bacterial transformations, calcium phosphate co-precipitation, electroporation, or liposome mediated-, DEAE dextran mediated-, polycationic mediated- or viral mediated transfection. (See, for example, Molecular Cloning: A Laboratory Manual (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press; Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed. (R.I. Freshney. 1987. Liss, Inc. New York, NY). A method of producing a polypeptide according to the invention is an additional part of the invention. The method comprises the steps of (a) culturing a host according to this aspect of the invention under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide.

[0157] In a further aspect, the invention provides an immunogenic composition comprising an effective amount of the nanostructure of any embodiment or combination of embodiments of the invention and a pharmaceutically acceptable carrier. The composition may comprise (a) a lyoprotectant; (b) a surfactant; (c) a bulking agent; (d) a tonicity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer.

[0158] In some embodiments, the buffer in the pharmaceutical composition is a Tris buffer, a histidine buffer, a phosphate buffer, a citrate buffer or an acetate buffer. The composition may also include a lyoprotectant, e.g. sucrose, sorbitol or trehalose. In certain embodiments, the composition includes a preservative e.g. benzalkonium chloride, benzethonium, chlorohexidine, phenol, m-cresol, benzyl alcohol, methylparaben, propylparaben, chlorobutanol, o-cresol, p-cresol, chlorocresol, phenylmercuric nitrate, thimerosal, benzoic acid, and various mixtures thereof. In other embodiments, the composition includes a bulking agent, like glycine. In yet other embodiments, the composition includes a surfactant e.g., polysorbate-20, polysorbate-40, polysorbate-60, polysorbate-65, polysorbate-80 polysorbate-85, poloxamer-188, sorbitan monolaurate, sorbitan monopalmitate, sorbitan monostearate, sorbitan monooleate, sorbitan trilaurate, sorbitan tristearate, sorbitan trioleaste, or a combination thereof. The composition may also include a tonicity adjusting agent, e.g., a compound that renders the formulation substantially isotonic or isoosmotic with human blood. Exemplary tonicity adjusting agents include sucrose, sorbitol, glycine, methionine, mannitol, dextrose, inositol, sodium chloride, arginine and arginine hydrochloride. In other embodiments, the composition additionally includes a stabilizer, e.g., a molecule which substantially prevents or reduces chemical and/or physical instability of the nanostructure, in lyophilized or liquid form. Exemplary stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

[0159] The nanostructure may be the sole active agent in the composition, or the composition may further comprise one or more other agents suitable for an intended use, including but not limited to adjuvants to stimulate the immune system generally and improve immune responses overall. Any suitable adjuvant can be used. The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. Exemplary adjuvants

include, but are not limited to, Adju-Phos™, Adjumer™ albumin-heparin microparticles, Algal Glucan, Algammulin, Alum, Antigen Formulation, AS-2 adjuvant, autologous dendritic cells, autologous PBMC, Avridine™, B7-2, BAK, BAY R1005, Bupivacaine, Bupivacaine-HCl, BWZL, Calcitriol, Calcium Phosphate Gel, CCR5 peptides, CFA, Cholera holotoxin (CT) and Cholera toxin B subunit (CTB), Cholera toxin A1-subunit-Protein A D-fragment fusion protein, CpG, CRL1005, Cytokine-containing Liposomes, D-Murapalmidine, DDA, DHEA, Diphtheria toxoid, DL-PGL, DMPC, DMPG, DOC/Alum Complex, Fowlpox, Freund's Complete Adjuvant, Gamma Inulin, Gerbu Adjuvant, GM-CSF, GMDP, hGM-CSF, hIL-12 (N222L), hTNF-alpha, IFA, IFN-gamma in pcDNA3, IL-12 DNA, IL-12 plasmid, IL-12/GM-CSF plasmid (Sykes), IL-2 in pcDNA3, IL-2/Ig plasmid, IL-2/Ig protein, IL-4, IL-4 in pcDNA3, Imiquimod™, ImmTher™, Immunoliposomes Containing Antibodies to Costimulatory Molecules, Interferon-gamma, Interleukin-1 beta, Interleukin-12, Interleukin-2, Interleukin-7, ISCOM(s)™, Iscoprep 7.0.3™, Keyhole Limpet Hemocyanin, Lipid-based Adjuvant, Liposomes, Loxoridine, LT(R192G), LT-OA or LT Oral Adjuvant, LT-R192G, LTK63, LTK72, MF59, MONTANIDE ISA 51, MONTANIDE ISA 720, MPL™, MPL-SE, MTP-PE, MTP-PE Liposomes, Murametide, Murapalmidine, NAGO, nCT native Cholera Toxin, Non-Ionic Surfactant Vesicles, non-toxic mutant E112K of Cholera Toxin mCT-E112K, p-Hydroxybenzoique acid methyl ester, pCIL-10, pCIL12, pCMVm-CAT1, pCMVN, Peptomer-NP, Pleuran, PLG, PLGA, PGA, and PLA, Pluronic L121, PMMA, PODDSTM, Poly rA: Poly rU, Polysorbate 80, Protein Cochleates, QS-21, Quadri A saponin, Quil-A, Rehydragel HPA, Rehydragel LV, RIBI, Ribiliike adjuvant system (MPL, TMD, CWS), S-28463, SAF-1, Sclavo peptide, Sendai Proteoliposomes, Sendai-containing Lipid Matrices, Span 85, Specol, Squalane 1, Squalene 2, Stearyl Tyrosine, Tetanus toxoid (TT), Theramide™, Threonyl muramyl dipeptide (TMDP), Ty Particles, and Walter Reed Liposomes. Selection of an adjuvant depends on the subject to be treated. Preferably, a pharmaceutically acceptable adjuvant is used.

[0160] In another aspect, the invention provides methods for generating an immune response to paramyxovirus and/or pneumovirus F protein in a subject, comprising administering to the subject an effective amount of the immunogenic composition of any embodiment or combination of embodiments of the invention to generate the immune response. In a further aspect, the invention provides methods for treating or preventing a paramyxovirus and/or pneumovirus infection in a subject, comprising administering to the subject an effective amount of the immunogenic composition of any embodiment or combination of embodiments of the invention, thereby treating or preventing paramyxovirus and/or pneumovirus infection in the subject.

[0161] In one embodiment, the paramyxovirus and/or pneumovirus comprises respiratory syncytial virus. "Respiratory Syncytial Virus" and "RSV" refer to a negative-sense, single-stranded RNA virus that causes a respiratory disease, especially in children. When the method comprises treating an RSV infection, the immunogenic compositions are administered to a subject that has already been infected with the RSV, and/or who is suffering from symptoms (including but not limited to lower respiratory tract infections, upper respiratory tract infections, bronchiolitis, pneumonia, fever, listlessness, diminished appetite, recurrent wheezing, and

asthma) indicating that the subject is likely to have been infected with the RSV. As used herein, “treat” or “treating” includes, but is not limited to accomplishing one or more of the following: (a) reducing paramyxovirus and/or pneumovirus titer in the subject; (b) limiting any increase of paramyxovirus and/or pneumovirus titer in the subject; (c) reducing the severity of paramyxovirus and/or pneumovirus symptoms; (d) limiting or preventing development of paramyxovirus and/or pneumovirus symptoms after infection; (e) inhibiting worsening of paramyxovirus and/or pneumovirus symptoms; (f) limiting or preventing recurrence of paramyxovirus and/or pneumovirus symptoms in subjects that were previously symptomatic for paramyxovirus and/or pneumovirus infection; and/or promoting maternal transmission of paramyxovirus and/or pneumovirus antibodies to infants (after maternal immunization).

[0162] When the method comprises limiting a paramyxovirus and/or pneumovirus infection, the immunogenic compositions are administered prophylactically to a subject that is not known to be infected, but may be at risk of exposure to the paramyxovirus and/or pneumovirus. As used herein, “limiting” means to limit RSV infection in subjects at risk of RSV infection. Groups at particularly high risk include children under age 18 (particularly infants 3 years or younger), adults over the age of 65, and individuals suffering from any type of immunodeficiency.

[0163] As used herein, an “effective amount” refers to an amount of the immunogenic composition that is effective for treating and/or limiting RSV infection. The immunogenic compositions are typically formulated as a pharmaceutical composition, such as those disclosed above, and can be administered via any suitable route, including orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intra-arterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. Polypeptide compositions may also be administered via microspheres, liposomes, immune-stimulating complexes (ISCOMs), or other microparticulate delivery systems or sustained release formulations introduced into suitable tissues (such as blood). Dosage regimens can be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). A suitable dosage range may, for instance, be 0.1 ug/kg-100 mg/kg body weight of the F protein or antigenic fragment thereof. The composition can be delivered in a single bolus, or may be administered more than once (e.g., 2, 3, 4, 5, or more times) as determined by attending medical personnel.

[0164] In one embodiment, the administering results in production of paramyxovirus and/or pneumovirus neutralizing antibodies in the subject. In another embodiment, the neutralizing antibodies are present in sera of the subject at a titer (1/ ID_{50}) of at least 1,000; in other embodiments, the neutralizing antibodies are present in sera of the subject at a titer of 2,000 or 5,000.

Examples

Methods:

Expression and Screening of Trimeric Building Blocks Comprising an F Protein and a Trimeric Assembly Domain

[0165] Human codon-optimized sequences for trimeric building blocks including and lacking DS-Cav1 fusions

were ordered from Genscript. Building blocks for single-component nanostructures (i.e., I3-01) were cloned into the pcDNA3.1 vector (ThermoFisher Scientific) containing one CMV promoter, while building blocks for two-component nanostructures (e.g., I53-50) were cloned into the pBudCE4.1™ vector (ThermoFisher Scientific) containing both CMV and EF-1 α promoters. Recombinant proteins were expressed by transient transfection of Expi293F™ cells (ThermoFisher Scientific) using polyethylenimine (PEI). Cell cultures were harvested five days post-transfection by centrifugation. Secreted proteins were analyzed by ELISA, using either direct coating of the cell supernatants or by sandwich ELISA. Briefly, 96-well MaxiSorp™ plates (Nunc) were coated with cell supernatant for direct ELISA or murine anti-His tag monoclonal antibody (ThermoFisher Scientific) for sandwich ELISA. Secreted proteins were detected using the human Palivizumab, MPE8, RSD5, and D25 monoclonal antibodies. Transfected Expi293F cells were fixed and permeabilized with BD cytofix/cytoperm (BD Biosciences), incubated with human Palivizumab, MPE8, and D25 monoclonal antibodies, and stained with Alexa Fluor 647-conjugated anti-human IgG antibody (Jackson ImmunoResearch). Stained cells were counted with a FACS Fortessa™ flow cytometer (BD Biosciences). Analysis was performed with FlowJo™ software. Cell lines were routinely tested for *mycoplasma* contamination.

Expression and Purification of DS-Cav1-I53-50A

[0166] Lentivirus was produced by transient transfection of 293T (ATCC) cells using linear 25-kDa polyethylenimine (PEI; Polysciences). Briefly, 4×10^6 cells were plated onto 10 cm tissue culture plates. After 24 h, 3 μ g of psPAX2, 1.5 μ g of pMD2G (Addgene™ plasmid #12260 and #12259, respectively) and 6 μ g of lentiviral vector plasmid were mixed in 500 μ l diluent (5 mM HEPES, 150 mM NaCl, pH=7.05) and 42 μ l of PEI (1 mg/ml) and incubated for 15 min. The DNA/PEI complex was then added to the plate drop-wise. Lentivirus was harvested 48 h post-transfection and concentrated 100-fold by low-speed centrifugation at 8000 g for 18 h. Transduction of the target cell line was carried out in 125 mL shake flasks containing 10×10^6 cells in 10 mL of growth media. 100 μ L of 100 \times lentivirus was added to the flask and the cells were incubated with shaking (225 rpm) at 37° C., in 8% CO₂ for 4-6 h. 20 mL of growth media was added to the shake flask after 4-6 h.

[0167] Transduced cells were expanded every other day to a density of 1×10^6 cells/ml until a final culture size of 4 L was reached. The media was harvested after 17 days of total incubation after measuring final cell concentration ($\sim 5 \times 10^6$ cells/mL) and viability ($\sim 90\%$ viable). Culture supernatant was harvested by low-speed centrifugation to remove cells from the supernatant. NaCl and NaN₃ were added to final concentrations of 250 mM and 0.02%, respectively. The supernatant was loaded over one 5 mL HisTrap™ FF Crude column (GE Healthsciences) at 5 ml/min by an AKTA Pure™ (GE Healthsciences). The nickel elution was applied to a HiLoad™ 16/600 Superdex 200 μ g column (GE Healthsciences) to further purify the target protein by size-exclusion chromatography. The size-exclusion purified target protein was snap frozen in liquid nitrogen and stored at -80° C.

In Vitro Assembly of DS-Cav1-Bearing Nanostructures

[0168] 100% valency particles (20 DS-Cav1 trimers per icosahedral nanostructure) were prepared by mixing

DS-Cav1-foldon-I53-50A trimers and I53-50B.4PT1 pentamers at 50 μM each and incubating with rocking overnight at 4° C. In some cases, assembled nanostructures were purified from excess components remaining in the in vitro assembly reaction using a GE Sephadryl S-500 HR 16/60 column in a buffer comprising 25 mM Tris pH 8, 250 mM NaCl, 5% glycerol. Sample load and SEC fractions were analyzed by SDS-PAGE in the presence and absence of reducing agent. Peak fractions were pooled, concentrated using a GE Vivaspin™ 20 30 kDa MWCO centrifugal filter, and quantified using an Agilent 8454 spectrophotometer. [0169] 66% valency particles (~14 DS-Cav1 trimers per icosahedral nanostructure) were prepared by mixing DS-Cav1-foldon-I53-50A trimers, I53-50A trimers, and I53-50B.4PosT1 pentamers at 50, 25, and 75 μM , respectively. 33% valency particles (~7 DS-Cav1 trimers per icosahedral nanostructure) were prepared by mixing DS-Cav1-foldon-I53-50A trimers, I53-50A trimers, and I53-50B.4PosT1 pentamers at 25, 50, and 75 μM , respectively. The in vitro assembly reactions were allowed to incubate with rocking overnight at 4° C. In some cases, assembled nanostructures were purified from excess components remaining in the in vitro assembly reaction using a GE Sephadryl™ S-500 HR 16/60 column in a buffer comprising 25 mM Tris pH 8, 250 mM NaCl, 5% glycerol. Sample load and SEC fractions were analyzed by SDS-PAGE in the presence and absence of reducing agent. Peak fractions were pooled, concentrated using a GE Vivaspin™ 20 30 kDa MWCO centrifugal filter, and quantified using an Agilent 8454 spectrophotometer after centrifuging at ~21,000 g for 10 minutes at 4° C. Samples were then transferred to cryogenic tubes in 1 mL aliquots at 1.1 mg/mL for the 33% valency particles and 0.6 mg/mL for the 66% valency particles, flash frozen in liquid nitrogen, and stored at -80° C.

Electron Microscopy of DS-Cav1-Bearing Nanostructures

[0170] Samples were prepared for negative stain EM by diluting to 0.01 mg/mL using 25 mM Tris pH 8, 250 mM NaCl, 5% glycerol and 3.5 μL was incubated on a glow-discharged, copper, carbon-coated grid for 20 seconds before blotting away the liquid with a piece of Whatman No. 1 filter paper. Within seconds of blotting away the sample, a 3.5 μL droplet of stain (2% w/v uranyl formate) was deposited and blotted away immediately, and then a second cycle of staining/blotting was performed.

Circular Dichroism (CD) Spectropolarimetry

[0171] CD spectra from F proteins (0.5 mg ml^{-1}) were recorded on a Chirascan™ spectropolarimeter (Applied Photophysics) over the wavelength range of 195 to 260 nm at a bandwidth of 1 nm, step size of 0.5 nm, and 1 s per step. The spectra in the far-ultraviolet region required an average of three scans and were subtracted from blank spectra performed with buffer. Thermal denaturation was monitored by performing scans at intervals of 1° C., after equilibration for 1 min at each temperature. Data were fitted to a simple first order curve. The values of AA222 are represented on the y axis as the percentage of the values recorded at 20° C.

Enzyme-Linked Immunosorbent Assay (ELISA)

[0172] To test specific binding of antibody or sera, 96-well MaxiSorp™ plates (Nunc) were coated with serial dilutions of tissue culture supernatants from cells expressing trimeric

building blocks comprising F proteins and a trimeric assembly domain or 2 $\mu\text{g ml}^{-1}$ of the following purified proteins: Ds-Cav1 with foldon, Ds-Cav1 fused to a trimeric first polypeptide or DS-Cav1-displaying nanostructures. Plates were blocked with 1% bovine serum albumin (BSA) and incubated with titrated antibodies (D25, MPE8, Palivizumab, RSD5) or murine sera followed by AP-conjugated goat anti-human IgG (Southern Biotech, 2040-04) or goat anti-mouse IgG (Southern Biotech, 1030-04). Plates were then washed with PBS buffer (Gibco, Invitrogen), 0.05% Tween-20 and substrate (p-NPP, Sigma) was added and plates were read at 405 nm.

Surface Plasmon Resonance (SPR)

[0173] The experiments were carried out at 25° C. on a ProteON™ XPR-36 instrument (Bio-Rad Laboratories) in a PBS buffer (Gibco, Invitrogen), 0.05% Tween-20. The D25 mAb was immobilized on a GLM sensor chip surface through amine coupling at 1000 response units (RU) and a blank surface with no protein was created under identical coupling conditions for use as a reference. Monoclonal antibodies (D25, MPE8, Palivizumab and 131-2a) were injected at a flow rate of 100 $\mu\text{l}/\text{min}$, at concentrations of 50 nM in different sensor channels. The data were processed using Proteon software and double referenced by subtraction of the blank surface and buffer only injection before local fitting of the data.

Vaccination and Serological Analysis

[0174] Female BALB/c mice 6-9 weeks of age were obtained from ENVIGO Laboratories (Italy). All proteins were formulated with AddaVax™ adjuvant (Invivogen) according to the manufacturer's instruction. Mice were immunized subcutaneously (s.c) with a total protein dose corresponding to 5 μg of the DS-Cav1 antigen equivalent on day 0, 14, and 28 in 50% AddaVax™ in PBS. Mice were bled on day 24 and 40. Recovered sera were used to measure binding and neutralizing titers. Binding titers were measured by coating 3 $\mu\text{g/ml}$ of DS-Cav1, I53-50 nanostructures or I53-50 nanostructure subunits.

Virus Neutralization Assay and Microscopy Analysis

[0175] Neutralization of RSV infection by sera was measured using a micro-neutralization flow cytometry-based assay. Serial dilutions of sera were pre-incubated with RSV for 1 hour at 37° C. and added to 10000 HEp-2 (ATCC® CCL-23™) cells/well in 96-well flat-bottom plates (MOI of 1). After 24 hours, cells were washed, detached and fixed with 2% formaldehyde. Percentage of GFP positive cells were measured by High throughput FACS with an Intellicyt coupled to an automated platform. The Tissue Culture Inhibiting Dilution (TCID) neutralizing 50% of the Infection (TCID₅₀) was calculated by nonlinear regression with Prism 7 (GraphPad Software).

Non-Human Primate (NHP) Immunization

[0176] Rhesus macaques were immunized i.m. (right quadriceps) at weeks 0 and 4 with trimeric DS-Cav1 (50 μg ; n=4) or DS-Cav1-foldon-I53-50 nanostructures (96 μg , comprising 50 μg of displayed DS-Cav1; n=5) formulated in the MF59-like adjuvant SWE. Sera were obtained at weeks 6 and 16 for serological analysis.

Stability of DS-Cav1-Bearing Nanostructures by Relative Binding to D25

[0177] Experiments were carried out at 20° C. on a ProteoN™ XPR-36 instrument (Bio-Rad Laboratories) in a PBS buffer (Gibco, Thermo Fisher Scientific) and 0.05% Tween-20 (Sigma). 100 nM D25 antibody was immobilized on a GLM sensor chip surface through amine coupling (EDC/NHS chemistry) and a blank surface with no antibody was created under identical coupling conditions for use as a reference. Analyte proteins (soluble DS-Cav1, soluble DS-Cav1-I53-50A and DS-Cav1-foldon-I53-50 nanostructures), heat stressed at different temperatures (20, 50, 70 or 80° C.) for 1 h, were injected at a flow rate of 100 µl/min, at a concentration of 50 nM in the different sensor channels. Data were processed using Proteon software and double referenced by subtraction of the blank surface and buffer-only injection before local fitting of the data.

Chemical Denaturation of Nanostructure-Related Proteins

[0178] Trimeric DS-Cav1, DS-Cav1-I53-50A, DS-Cav1-I53-50, I53-50, trimeric I53-50A, or pentameric I53-50B.4PT1 was diluted to a final concentration of 2.5 µM in 25 mM Tris pH 8, 250 mM NaCl, 5% glycerol with varying concentrations of guanidine hydrochloride, ranging from 0 M to 6.5 M, increasing in 0.25 M increments. Samples were prepared in triplicate and incubated for 16 hours at ambient temperature. On a Cary Eclipse Fluorescence Spectrophotometer, intrinsic fluorescence was measured for each guanidine hydrochloride concentration of each protein and of each replicate. A Peltier controller was used in the cell holder to maintain a temperature of 25° C. throughout all experiments. Using a 10 mm cell (Agilent Cuvette, part #6610021600), fluorescence spectra were collected, exciting at 290 nm and scanning emission from 310 nm to 510 nm at a rate of 60 nm/minute with a bandpass of 1 nm.

Statistical Analysis

[0179] No statistical methods were used to predetermine sample size. Data were analyzed with Prism 6 (GraphPad™ Software) using the two-tailed non-parametric Mann-Whitney U test for two groups' comparison, or Kruskall-Wallis test (and Dunn's posttest) when three or more groups were compared.

Results

Trimeric Building Blocks Comprising an F Protein and a Trimeric Assembly Domain

[0180] Several trimeric building blocks, each comprising an F protein genetically fused to a trimeric assembly domain, were found to be secreted from HEK293F cells with their F proteins in a well-folded, prefusion conformation as judged by prefusion-specific monoclonal antibody binding in ELISA assays. FIG. 2 shows an example of ELISA data analyzing the supernatant of HEK293F cells expressing DS-Cav1-foldon, DS-Cav1-foldon-T33-31A, and DS-Cav1-T33-31A. Several other trimeric building blocks yielded detectable secretion of well-folded, prefusion F proteins.

Expression and Purification of DS-Cav1-Foldon-I53-50A

[0181] A lentiviral vector encoding DS-Cav1-foldon-I53-50A was used to transduce HEK293F cells for large-scale

expression. The secreted protein was purified from tissue culture supernatants by immobilized metal affinity chromatography and size exclusion chromatography. Size exclusion chromatograms (FIG. 3) indicated that the purified protein formed a single, monodisperse species.

Expression and Purification of I53-50B.4PT1

[0182] I53-50B.4PT1, a pentameric protein comprising a second assembly domain that interacts with the trimeric assembly domain in I53-50A or DS-Cav1-foldon-I53-50A to drive assembly of icosahedral I53-50-based nanostructures, was expressed and purified as described in Bale et al. and patent publication US20160122392 A1.

In Vitro Assembly and Characterization of DS-Cav1-Bearing I53-50 Nanostructures

[0183] I53-50 is a 120-subunit two-component nanostructure with icosahedral symmetry comprising 20 trimeric (I53-50A) and 12 pentameric (I53-50B) building blocks, as recently described by Bale et al. The N terminus of I53-50A is exposed on the exterior of the I53-50 nanostructure, which enables the display of antigens on the nanostructure exterior through genetic fusion to the I53-530A N terminus. Purified DS-Cav1-foldon-I53-50A and I53-50B.4PT1 were assembled in vitro to form 120-subunit icosahedral nanostructures displaying various amounts of DS-Cav1 on the nanostructure exteriors by mixing the two purified proteins in various molar ratios. In separate preparations, nanostructures displaying DS-Cav1 at valencies of 100% (20 trimers), 66% (~14 trimers), and 33% (~7 trimers) were prepared as described above. The species present in the in vitro assembly reactions after overnight incubation were assessed by several techniques, including size exclusion chromatography-multi-angle light scattering (SEC-MALS), dynamic light scattering, and UV/vis spectroscopy. Assembled, 120-subunit nanostructures were purified from the in vitro assembly reactions using size exclusion chromatography (an example chromatogram obtained using the 100% valency nanostructures is presented in FIG. 4). The purified nanostructures were characterized by negative stain electron microscopy, which revealed fields of monodisperse particles in which DS-Cav1 was clearly visible as spikes projecting outward from the core icosahedral I53-50 assembly (an example micrograph obtained using the 100% valency particles is presented in FIG. 5). ELISA assays using monoclonal antibodies specific to the prefusion conformation confirmed that the DS-Cav1 thus displayed on the nanostructure exteriors was well-folded and antigenically intact (FIG. 6). Surface plasmon resonance experiments evaluating the kinetics of monoclonal antibody binding revealed that antibody dissociation from the 100% valency DS-Cav1-foldon-I53-50 nanostructures was slower than from DS-Cav1-foldon trimers, likely due to avidity effects deriving from the multivalent presentation of DS-Cav1 on the nanostructure exterior (FIG. 6). Together, these experiments confirmed that the DS-Cav1-foldon-I53-50 nanostructures formed monodisperse, icosahedral nanostructures that display well-folded, antigenically intact DS-Cav1 trimers on their exteriors. These findings motivated experiments to evaluate the utility of the DS-Cav1-foldon-I53-50 nanostructures as immunogens for inducing humoral immune responses against DS-Cav1 in animals.

Immunogenicity of DS-Cav1-Foldon-I53-50 Nanostructures

[0184] The DS-Cav1-foldon-I53-50 nanostructures displaying DS-Cav1 at 33%, 66%, and 100% valency were injected into mice using a prime-boost strategy as described above. Additional groups of mice were injected with trimeric DS-Cav1-foldon as a benchmark for the humoral immune response induced against DS-Cav1 by the nanostructures or I53-50 nanostructures lacking displayed DS-Cav1 as negative controls for a DS-Cav1 specific response. ELISA assays of serum extracted from the mice at defined time points after the injections were used to measure DS-Cav1 specific antibody titers present in the sera of the injected animals (FIG. 7). As expected, sera from animals injected with the I53-50 nanostructures lacking displayed DS-Cav1 did not contain antibodies specific to DS-Cav1. Trimeric DS-Cav1-foldon induced DS-Cav1-specific antibodies, in accordance with previous results (McClellan et al.). The 33%, 66%, and 100% valency DS-Cav1 nanostructures all induced higher DS-Cav1-specific antibody titers than trimeric DS-Cav1-foldon, with the antibody titers increasing with increasing DS-Cav1 valency. DS-Cav1-specific titers were roughly 2.5-fold higher on average in mice injected with 100% valency DS-Cav1-foldon-I53-50 nanostructures compared to DS-Cav1. These results demonstrate that immunogens in which paramyxovirus F proteins are multivalently displayed on self-assembling protein nanostructures can induce higher humoral immune responses when injected into animals.

[0185] The sera from the mice injected with the series of immunogens described above was also evaluated for the presence of neutralizing antibody titers using the standard neutralization assay in HEp-2 cells (FIG. 8). The trend in serum neutralizing antibody titers correlated highly with the trend observed in DS-Cav1-specific binding antibody titers. Sera from animals injected with the I53-50 nanostructures lacking displayed DS-Cav1 did not neutralize virus, consistent with the lack of DS-Cav1-specific antibodies in these sera. The sera from animals injected with trimeric DS-Cav1-foldon neutralized virus with an average titer ($1/\text{ID}_{50}$) of 3,030. The 33%, 66%, and 100% valency DS-Cav1-I53-50 nanostructures induced higher neutralizing antibody titers than trimeric DS-Cav1-foldon, with average titers of 9,400, 20,000, and 30,500, respectively. These results demonstrate that the higher humoral response induced by immunogens in which paramyxovirus F proteins are multivalently displayed on self-assembling protein nanostructures result in more effective virus neutralization.

[0186] The DS-Cav1-foldon-I53-50 nanostructures were also injected into Rhesus macaques to evaluate their immunogenicity in a primate immune system. The animals were injected intramuscularly at weeks 0 and 4 with either free DS-Cav1 trimer or DS-Cav1-foldon-I53-50 nanostructures displaying DS-Cav1 at 100% valency. In both cases, the dose of DS-Cav1 antigen was 50 µg, and the immunogens were formulated with the MF59-like, squalene-based oil-in-water emulsion adjuvant SWE. Sera obtained from the animals at weeks 6 and 16 were evaluated for anti-DS-Cav1 antibody titers and RSV-neutralizing antibody titers (FIG. 9). The results mirrored those obtained in mice. At week 16, the mean anti-DS-Cav1 antibody titer was 4-fold higher in animals injected with the DS-Cav1-foldon-I53-50 nanostructure compared to animals injected with trimeric DS-Cav1. The mean RSV-neutralizing antibody titer at week 16 was 16-fold higher in animals injected with the DS-Cav1-

foldon-I53-50 nanostructure compared to animals injected with trimeric DS-Cav1. These results demonstrate, in a primate immune system, that immunogens in which paramyxovirus F proteins are multivalently displayed on self-assembling protein nanostructures induce more robust humoral immune responses, including high levels of virus-neutralizing antibodies, than the trimeric paramyxovirus F proteins alone.

Physical Stabilization of DS-Cav1 by Fusion to I53-50A

[0187] Given the key antigenic properties of prefusion F, we used two orthogonal approaches to measure the physical stability of DS-Cav1 when fused to I53-50A and/or when further assembled into the icosahedral nanostructure. The first assay measured the retention of binding by a prefusion-specific mAb (D25) after thermal stress, an approach that has been used previously to characterize prefusion F stability (McClellan et al. 2013; Joyce et al. 2016; Krarup et al. 2015). Samples of trimeric DS-Cav1, trimeric DS-Cav1-I53-50A, and DS-Cav1-I53-50 nanostructures containing equivalent concentrations (50 nM) of DS-Cav1 were split into four aliquots and incubated at 20, 50, 70 or 80° C. for 1 hour. After cooling to room temperature, D25 binding was assayed by surface plasmon resonance (SPR). We found that all samples bound D25 equivalently at 20 and 50° C., but lost most of their reactivity to D25 after 1 hour at 80° C. as previously reported for DS-Cav1 (McClellan et al. 2013; Joyce et al. 2016) (FIG. 10). Interestingly, while D25 was also unable to bind trimeric DS-Cav1 incubated at 70° C. for 1 hour, trimeric DS-Cav1-I53-50A and the DS-Cav1-I53-50 nanostructures retained 50 and 80% of their respective binding signals (FIG. 10). While the multivalent nature of the DS-Cav1-I53-50 nanostructures complicates direct quantitative comparisons to trimeric DS-Cav1, these results indicate that genetic fusion to the I53-50A trimer further stabilizes the prefusion conformation of DS-Cav1, and suggest that this increased stability is maintained in the context of the assembled nanostructure immunogen.

[0188] We used chemical denaturation in guanidine hydrochloride (GdnHCl), monitored by intrinsic tryptophan fluorescence, as a second, antibody-independent technique to evaluate physical stability. Analyzing fluorescence emission from DS-Cav1 incubated in 0-6.5 M GdnHCl revealed that the protein undergoes two subtly distinct transitions, one between 0.25 and 2.25 M GdnHCl and another between 2.25 and 5.75 M (FIG. 11). In contrast, only a single transition is apparent for trimeric DS-Cav1-I53-50A, occurring between 2.25 and 6.25 M GdnHCl (FIG. 11). It is unclear at present whether the transition at lower [GdnHCl] observed for DS-Cav1 is absent from trimeric DS-Cav1-I53-50A or simply shifted to higher [GdnHCl]. However, it is clear that the native conformation of DS-Cav1 is stabilized by genetic fusion to trimeric I53-50A, mirroring the results obtained by measuring D25 binding after thermal stress. Comparing the data for the DS-Cav1-I53-50 nanostructure and the I53-50 nanostructure alone (lacking fused DS-Cav1) indicated that the stabilization is maintained upon assembly to the icosahedral nanostructure (FIG. 11). The source of this effect is likely the extreme stability of the I53-50A trimer. I53-50A is derived from the KDPG aldolase of the hyperthermophilic bacterium *T. maritima* and only began to exhibit changes in fluorescence at very high (5.75 M) GdnHCl concentrations (FIG. 11).

[0189] We made addition constructs to assess the number of GS repeats and the need for a stabilization domain such as the Foldon moiety.

Sequence Information

IPD Name	MS (Da)	Construct Information
RSV_F-10	74005.38	DS-Cav1-8GS-HelExt-50A
RSV_F-11	74293.64	DS-Cav1-12GS-HelExt-50A
RSV_F-12	74551.87	DS-Cav1-16GS-HelExt-50A
RSV_F-13	77212.97	DS-Cav1-foldon-10GS-HelExt-50A
RSV_F-14	77558.28	DS-Cav1-foldon-15GS-HelExt-50A
RSV_F-15	77933.62	DS-Cav1-foldon-20GS-HelExt-50A

[0190] Studies were based on expression yield in a small-scale transient transfection. Plasmids capable of expressing the relevant constructs were transformed into NEB 5u *E. coli* cells and selected on LB+carbenicillin agar plates. 1 mL cultures were prepared by inoculating TB media with a bacterial colony and again selecting with 50 µg/mL carbenicillin. A Qiagen Mini Prep kit was used to purify plasmid from the *E. coli* cultures in accordance with their protocol. Expi293FT™ Cells (ThermoFisher) were cultured in Expi293™ Expression Medium (ThermoFisher) supplemented with penicillin (100 u/mL) and streptomycin (100 µg/mL) at 8% CO₂, 37° C., and 125 rpm shaking.

[0191] On the day prior to transfection, cells were seeded at a concentration of 2E6 cells/mL. On the day of transfection, cells were counted by a Countess II (ThermoFisher) with trypan blue to determine cell viability. Cell concentration was adjusted to 2.5E6 cells/mL, and cells were plated into untreated 12-well plates (Corning) in 1 mL volumes. 1 µg of DNA plasmid were transfected per each well using Expifectamine™ (ThermoFisher), following the manufacturer's directions. Enhancers, components of ThermoFis-

ther's Expifectamine™ Transfection Kit, were added 18 hours after transfection. The 1 mL cultures were harvested 5 days post-transfection, and the cells were pelleted from the supernatant by centrifugation at 1,500xg for 5 minutes at 4° C. Supernatants were filtered through a 0.45 µM filter with a PVDF membrane.

[0192] Filtered supernatants containing DS-Cav1-I53-50A constructs were denatured and boiled for 10 minutes at 95° C. for 10 minutes in 2x Laemmli buffer with 2-mercaptoethanol. SDS-PAGE separated the sample fractions, which were then transferred to a nitrocellulose membrane and probed with palivizumab, followed with a secondary antibody, anti-human conjugated to HRP. Blot was imaged using Clarity Western ECL Blotting Substrate (Bio-Rad).

[0193] Filtered supernatants containing DS-Cav1-I53-50A constructs were bound to Nunc MaxiSorp™ 96-well plates in a two-fold dilution series. The pre-fusion conformation-specific antibody D25 was used to detect DS-Cav1-I53-50A, followed by a secondary anti-human antibody conjugated to HRP. Protein yield was determined colorimetrically via the substrate TMB and absorbances were collected at 450 nm.

[0194] The expression yields and binding of the prefusion-specific mAb D25 (data not shown) indicate that all constructs express well and are in the prefusion conformation. Those of skill in the art would have expected that a heterologous trimerization domain (such as the foldon) would be required for proper expression and folding of prefusion F constructs. Our results indicate that the I53-50A nanostructure component can support the expression and proper folding of DS-Cav1 without the use of a trimerization domain like the foldon. Binding of D25 to these constructs suggests that they are antigenically intact and would be expected to induce potent immune responses, including neutralizing antibodies, similarly to nanostructures comprising the DS-Cav1-foldon-I53-50 fusion polypeptide.

SEQUENCE LISTING

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organism = synthetic construct

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LALGLSALKF FPAEPFQGVRL VLRAYAEVFP EVRFLPTGGI KEEHLPHYAA LPNLLAVGGS 180
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organism = synthetic construct

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organism = synthetic construct

SEQUENCE: 16
MGANWYLDNE SSRLSFTSTK NADIAEVHRF LVLHGKVDPK GLAEVEVETE SISTGIPLRD 60
MLLRVILVFQV SKFPVQAQINA QLDMRPINNL APGAQLELRL PLTVSLRGKS HSYNAELLAT 120
RLLDERRFQVV TLEPPLVIHAQ DFDMVRAFNA LRLVAGLSAV SLSVPVGAVL IFTAR 175

SEQ ID NO: 17          moltype = AA length = 208
FEATURE
REGION
1..208
note = I32-06A
VARIANT
1
note = optionally absent
source
1..208
mol_type = protein
organism = synthetic construct

SEQUENCE: 17
MTDYIRDGSA IKALSPFAIL AEADLRRHIPO DLQRЛАVRVI HACGMV рDAN DLAFSEGAGK 60
AGRНALLAGA PILCDARMVA EGITRSRLPA DNРVIYTЛSD PSVPELAKKI GNTRSAALD 120
LWLPHIEGSI VAIGNAPTAL FRLFELLPDA APKPALIIGM PVGFVGAAES KDELAANSRG 180
VPYVIVRGRR GGSAMTAAAV NALASERE 208

SEQ ID NO: 18          moltype = AA length = 128
FEATURE
REGION
1..128
note = I32-06B
VARIANT
1
note = optionally absent
source
1..128
mol_type = protein
organism = synthetic construct

SEQUENCE: 18
MITVFGLKSK LAPRREKLAЕ VIYSSLHLGL DIPKGKHAIR FLCLEKEDFY YPFDRSDDYT 60
VIEINLMAGR SEETKMLLIF LLFIALERKL GIRAHDVEIT IKEQPAHCWG FRGRTGDSAR 120
DLDYDIYV 128

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SEQ ID NO: 19      moltype = AA length = 235
FEATURE          Location/Qualifiers
REGION           1..235
note = I32-19A
VARIANT          1
note = optionally absent
source           1..235
mol_type = protein
organism = synthetic construct

SEQUENCE: 19
MGSDLQKLQR FSTCDISDGL LNVYNIPTGG YFPNLTAISP PQNSSIVGTA YTVLFAPIDD 60
PRPAVNYIDS VPPNSILVLA LEPHLQSQFH PFIKIQAMY GGLMSTRAQY LKSNGTVVFG 120
RIRDVDEHRT LNHPVFAYGV GSCAPKAVVK AVGTNVQLKI LTSDDGVTQTI CPGDYIAGDN 180
NGIVRIPVQE TDISKLVTYI EKSIEVDRLV SEAIKNGLPA KAAQTARRMV LKDVI 235

SEQ ID NO: 20      moltype = AA length = 162
FEATURE          Location/Qualifiers
REGION           1..162
note = I32-19B
VARIANT          1
note = optionally absent
source           1..162
mol_type = protein
organism = synthetic construct

SEQUENCE: 20
MSGMRVYLGA DHAGYELKQA IIAPFLKMTGH EPIDCGALRY DADDDYPAPFC IAAATRTVAD 60
PGSLGIVLGG SGNGEQIAAN KVPGARCALA WSVQTAALAR EHNNNAQLIGI GGRMHTLEEA 120
LRIVKAFVTT PWSKAQRHQR RIDILAEYER THEAPPVPGA PA 162

SEQ ID NO: 21      moltype = AA length = 157
FEATURE          Location/Qualifiers
REGION           1..157
note = I32-28A
VARIANT          1
note = optionally absent
source           1..157
mol_type = protein
organism = synthetic construct

SEQUENCE: 21
MGDDARIAAI GDVDELNSQI GVLLAEPLPD DVRAALSAIQ HDLFDLGGEL CIPGHAITE 60
DHLLRLALWL VHYNGQLPPL EEFILPGGAR GAALAHVCRT VRRAERSIK ALGASEPLNI 120
APAAYVNLLS DLLFVLARVL NRAAGGADVL WDRTRAH 157

SEQ ID NO: 22      moltype = AA length = 157
FEATURE          Location/Qualifiers
REGION           1..157
note = I32-28B
VARIANT          1
note = optionally absent
source           1..157
mol_type = protein
organism = synthetic construct

SEQUENCE: 22
MILSAEoSFT LRPHHGAAA LAFVREPAAA LAGVQRLRGL DSDGEQVWGE LLVRVPLLGE 60
VDLPRSEIV RTPQGAELRP LTLTGERAWV AVSGQATAAE GGEMAFAFQF QAHLATPEAE 120
GEGGAAFEVMM VQAAAGVTLL LVAMALPQGL AAGLPPA 157

SEQ ID NO: 23      moltype = AA length = 156
FEATURE          Location/Qualifiers
REGION           1..156
note = I53-40A.1
VARIANT          1
note = optionally absent
source           1..156
mol_type = protein
organism = synthetic construct

SEQUENCE: 23
MTKKVGIVDT TFARVDMASA AILTLKMKESP NIKIIRKTVP GIKDLPVACK KLLEEGCDI 60
VMALGMPGKK EKDKVCAHEA SLGLMLAQLM TNKHIIEVFF HEDEAKDDAE LKILAARRAI 120
EHALNVYYLL FKPEYLTRMA GKGLRQGFED AGPARE 156

SEQ ID NO: 24      moltype = AA length = 209
FEATURE          Location/Qualifiers
REGION           1..209
note = I53-40B.1

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VARIANT           1
source          note = optionally absent
                1..209
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 24
MDDINNQLKR LKVIPVIAID NAEDIIPLGK VLAENGLPAA EITFRSSAAV KAIMLLRSAQ 60
PEMLIGAGTI LNGVQALAAK EAGADFVVSP GFNPNTVRAC QIIGIDIVPG VNNPSTVEQA 120
LEMGLTTLKF FPAEASGGIS MVKSLVGPYG DIRLMPTGGI TPDNIDNYLA IPQVLACGGT 180
WMVDKKLVRN GEWDEIARLT REIVEQVNP 209

SEQ ID NO: 25      moltype = AA length = 114
FEATURE          Location/Qualifiers
REGION           1..114
source          note = I53-47A.1
                1
                note = optionally absent
                1..114
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 25
MPIFTLNTNI KADDVPSDFL SLTSRLVGLI LSKPGSYVAV HINTDQQLSF GGSTNPAAFG 60
TLMSIGGIEP DKNRDHSAVL FDHLNAMLGI PKNRMYIHFV NLNGDDVGWN GTTF 114

SEQ ID NO: 26      moltype = AA length = 114
FEATURE          Location/Qualifiers
REGION           1..114
source          note = I53-47A.1NegT2
                1
                note = optionally absent
                1..114
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 26
MPIFTLNTNI KADDVPSDFL SLTSRLVGLI LSEPGSYVAV HINTDQQLSF GGSTNPAAFG 60
TLMSIGGIEP DKNRDHSAVL FDHLNAMLGI PKNRMYIHFV DLDGDDVGWN GTTF 114

SEQ ID NO: 27      moltype = AA length = 157
FEATURE          Location/Qualifiers
REGION           1..157
source          note = I53-47B.1
                1
                note = optionally absent
                1..157
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 27
MNQHSHKDHE TVRIAVVRAR WHADIVDACV EAIFIAMAAI GGDRFAVDVF DVPGAYE IPL 60
HARTLAETGR YGAVLGTAFV VNGGIYRHEF VASAVIDGMN NVQLDTGVPV LSAVLTPHY 120
RDSDEHHRRF AAHFAVKGVE AARACIEILN AREKIAA 157

SEQ ID NO: 28      moltype = AA length = 157
FEATURE          Location/Qualifiers
REGION           1..157
source          note = I53-47B.1NegT2
                1
                note = optionally absent
                1..157
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 28
MNQHSHKDHE TVRIAVVRAR WHADIVDACV EAIFIAMAAI GGDRFAVDVF DVPGAYE IPL 60
HARTLAETGR YGAVLGTAFV VDGIIYDHEF VASAVIDGMN NVQLDTGVPV LSAVLTPHEY 120
EDSDEDHEFF AAHFAVKGVE AARACIEILN AREKIAA 157

SEQ ID NO: 29      moltype = AA length = 205
FEATURE          Location/Qualifiers
REGION           1..205
source          note = I53-50A.1
                1
                note = optionally absent
                1..205
                mol_type = protein
                organism = synthetic construct
SEQUENCE: 29
MKMEELFKKH KIVAVLRANS VEEAIEKAVA VFAGGVHLIE ITFTVPDADT VIKALSVLKE 60

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KGAIIGAGTV TSVEQCRKAV ESGAEFIVSP HLDEEISQFC KEKGVFYMPG VMTPTELVKA	120
MKLGHDLILKL FPGEVVGPQF VKAMKGPFPN VKFVPTGGVN LDNVCEWFKA GVLAVGVGDA	180
LVKGDPDEVR EAKKKPVEKI RGCTE	205
SEQ ID NO: 30 moltype = AA length = 205	
FEATURE Location/Qualifiers	
REGION 1..205	
VARIANT note = I53-50A.1NegT2	
1	
note = optionally absent	
source 1..205	
mol_type = protein	
organism = synthetic construct	
SEQUENCE: 30	
MKMEELFKKH KIVAVLRLANS VEEAIEKAVA VFAGGVHLIE ITFTVPDADT VIKALSVLKE	60
KGAIIGAGTV TSVEQCRKAV ESGAEFIVSP HLDEEISQFC KEKGVFYMPG VMTPTELVKA	120
MKLGHDLILKL FPGEVVGPQF VEAMKGPFPN VKFVPTGGVD LDDVCEWFDA GVLAVGVGDA	180
LVEGDPDEVR EDAKEPVEEI RGCTE	205
SEQ ID NO: 31 moltype = AA length = 205	
FEATURE Location/Qualifiers	
REGION 1..205	
VARIANT note = I53-50A.1Post1	
1	
note = optionally absent	
source 1..205	
mol_type = protein	
organism = synthetic construct	
SEQUENCE: 31	
MKMEELFKKH KIVAVLRLANS VEEAIEKAVA VFAGGVHLIE ITFTVPDADT VIKALSVLKE	60
KGAIIGAGTV TSVEQCRKAV ESGAEFIVSP HLDEEISQFC KEKGVFYMPG VMTPTELVKA	120
MKLGHDLILKL FPGEVVGPQF VKAMKGPFPN VKFVPTGGVN LDNVCKWFKA GVLAVGVGKA	180
LVKGKPDEV R EAKKKPVKKI RGCTE	205
SEQ ID NO: 32 moltype = AA length = 157	
FEATURE Location/Qualifiers	
REGION 1..157	
VARIANT note = I53-50B.1	
1	
note = optionally absent	
source 1..157	
mol_type = protein	
organism = synthetic construct	
SEQUENCE: 32	
MNQHSHKDHE TVRIAVVRAR WHAEIVDACV SAFEAMRDI GGDRFAVDVF DVPGAYE IPL	60
HARTLAETGR YGAVLGTA FV VNNGGIYRHEF VASAVIDGMM NVQLDTGVPV LSAVLTPHRY	120
RDSDAHTLLF LALFAVKGM E AARACVEILA AREKIAA	157
SEQ ID NO: 33 moltype = AA length = 157	
FEATURE Location/Qualifiers	
REGION 1..157	
VARIANT note = I53-50B.1NegT2	
1	
note = optionally absent	
source 1..157	
mol_type = protein	
organism = synthetic construct	
SEQUENCE: 33	
MNQHSHKDHE TVRIAVVRAR WHAEIVDACV SAFEAMRDI GGDRFAVDVF DVPGAYE IPL	60
HARTLAETGR YGAVLGTA FV VDGGIYDHEF VASAVIDGMM NVQLDTGVPV LSAVLTPHEY	120
EDSDADTLLF LALFAVKGM E AARACVEILA AREKIAA	157
SEQ ID NO: 34 moltype = AA length = 157	
FEATURE Location/Qualifiers	
REGION 1..157	
VARIANT note = I53-50B.4Post1	
1	
note = optionally absent	
source 1..157	
mol_type = protein	
organism = synthetic construct	
SEQUENCE: 34	
MNQHSHKDHE TVRIAVVRAR WHAEIVDACV SAFEAMRDI GGDRFAVDVF DVPGAYE IPL	60
HARTLAETGR YGAVLGTA FV VNNGGIYRHEF VASAVINGMM NVQLNTGVPV LSAVLTPHNY	120
DKSKAHTLLF LALFAVKGM E AARACVEILA AREKIAA	157

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SEQ ID NO: 35      moltype = AA length = 156
FEATURE          Location/Qualifiers
REGION           1..156
note = I53-40 A genus
VARIANT          1
note = optionally absent
VARIANT          70
note = Xaa is A or K
source           1..156
mol_type = protein
organism = synthetic construct
SEQUENCE: 35
MTKKVGIVDT TFARVDMASA AILTLKMESP NIKIIRKTVP GIKDLPVACK KLLEEGCDI 60
VMALGMPGKX EKDKVCAHEA SLGLMLAQLM TNKHIIEVFV HEDEAKDDAE LKILAARRAI 120
EHALNVYYLL FKPEYLTRMA GKGLRQGFED AGPARE                         156

SEQ ID NO: 36      moltype = AA length = 209
FEATURE          Location/Qualifiers
REGION           1..209
note = I53-40 B genus
VARIANT          1
note = optionally absent
VARIANT          2
note = Xaa is S or D
VARIANT          3
note = Xaa is T or D
VARIANT          10
note = Xaa is A or R
VARIANT          85
note = Xaa is T or D
VARIANT          119
note = Xaa is A or Q
VARIANT          163
note = Xaa is S or D
VARIANT          189
note = Xaa is T or R
source           1..209
mol_type = protein
organism = synthetic construct
SEQUENCE: 36
MXXINNQLKX LKVIPVIAID NAEDIIPLGK VLAENGLPAA EITFRSSAAV KAIMLLRSAQ 60
PEMLIGAGTI LNGVQALAAK EAGAXFVVSP GFNPNTVRAC QIIGIDIVPG VNNPSTVEXA 120
LEMGLTTLKF FPAEASGGIS MVKSLVGPYG DIRLMPTGGI TPXNIDNYLA IPQVLACGGT 180
WMVDKKLVNX GEWDEIARLT REIVEQVNP                         209

SEQ ID NO: 37      moltype = AA length = 114
FEATURE          Location/Qualifiers
REGION           1..114
note = I53-47A genus
VARIANT          1
note = optionally absent
VARIANT          13
note = Xaa is T or D
VARIANT          33
note = Xaa is K or E
VARIANT          71
note = Xaa is S or D
VARIANT          74
note = Xaa is R or E
VARIANT          101
note = Xaa is N or D
VARIANT          103
note = Xaa is N or D
source           1..114
mol_type = protein
organism = synthetic construct
SEQUENCE: 37
MPIFTLNNTNI KAXDVPSDFL SLTSRLVGLI LSXPGSYAV HINTDQQLSF GGSTNPAAFG 60
TLMSIGGIEP XKNXDHSAVL FDHHLNAMLGI PKNRMYIHFF XLXGDDVGWN GTTF     114

SEQ ID NO: 38      moltype = AA length = 157
FEATURE          Location/Qualifiers
REGION           1..157
note = I53-47B genus
VARIANT          1
note = optionally absent

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VARIANT          9
VARIANT          note = Xaa is Y or H
VARIANT          82
VARIANT          note = Xaa is N or D
VARIANT          87
VARIANT          note = Xaa is R or D
VARIANT          105
VARIANT          note = Xaa is S or D
VARIANT          119
VARIANT          note = Xaa is R or E
VARIANT          121
VARIANT          note = Xaa is R or E
VARIANT          124
VARIANT          note = Xaa is A or D
VARIANT          126
VARIANT          note = Xaa is H or D
VARIANT          128
VARIANT          note = Xaa is R or E
VARIANT          150
VARIANT          note = Xaa is A or N
source           1..157
mol_type = protein
organism = synthetic construct

SEQUENCE: 38
MNQHSHKDXE TVRIAVVRAR WHADIVDACV EAFEIAMAII GGDRFAVDVF DVPGAYEIPL 60
HARTLAETGR YGAVLGTAFV VXXGIYXHEF VASAVIDGMN NVQLXTGVPV LSAVLTPHXY 120
KDSXEXHXFF AAHFAVKGVE AARACIEILX AREKIAA 157

SEQ ID NO: 39      moltype = AA length = 205
FEATURE          Location/Qualifiers
REGION           1..205
VARIANT          note = I53-50A genus
VARIANT          1
VARIANT          note = optionally absent
VARIANT          126
VARIANT          note = Xaa is T or D
VARIANT          139
VARIANT          note = Xaa is Q or E
VARIANT          142
VARIANT          note = Xaa is K or E
VARIANT          160
VARIANT          note = Xaa is N or D
VARIANT          163
VARIANT          note = Xaa is N or D
VARIANT          166
VARIANT          note = Xaa is E or K
VARIANT          169
VARIANT          note = Xaa is D or K
VARIANT          179
VARIANT          note = Xaa is S, D or K
VARIANT          183
VARIANT          note = Xaa is K or E
VARIANT          185
VARIANT          note = Xaa is T, D, or K
VARIANT          192
VARIANT          note = Xaa is D or K
VARIANT          195
VARIANT          note = Xaa is A, E, or K
VARIANT          198
VARIANT          note = Xaa is E or K
VARIANT          199
VARIANT          note = Xaa is E or K
source           1..205
mol_type = protein
organism = synthetic construct

SEQUENCE: 39
MKMEELFKKH KIVAVLTRANS VEEAIEKAVA VFAGGVHLIE ITFTVPDADT VIKALSVLKE 60
KGAIIGAGTV TSVEQCRKAV ESGAEFIVSP HLDEEISQFC KEKGVFYMPG VMTPTELVKA 120
MKGHGXILKL FPGEVVGPXF VXAMKGPFPN VKFPVPTGGVX LDXVCXWFXA GVLAVGVGX 180
LVXGXPDEVR EXAKXFPXXI RGCTE 205

SEQ ID NO: 40      moltype = AA length = 157
FEATURE          Location/Qualifiers
REGION           1..157
VARIANT          note = I53-50B genus
VARIANT          1

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VARIANT          note = optionally absent
                 9
VARIANT          note = Xaa is Y or H
                 38
VARIANT          note = Xaa is A or R
                 82
VARIANT          note = Xaa is N or D
                 87
VARIANT          note = Xaa is R or D
                 97
VARIANT          note = Xaa is N or D
                 105
VARIANT          note = Xaa is S, N, or D
                 119
VARIANT          note = Xaa is R, E, or N
                 121
VARIANT          note = Xaa is R, E, or D
                 122
VARIANT          note = Xaa is K or D
                 124
VARIANT          note = Xaa is K or D
                 126
VARIANT          note = Xaa is H or D
source           1..157
                 mol_type = protein
                 organism = synthetic construct

SEQUENCE: 40
MNQHSHKDXE TVRIAVVRAR WHAEIVDACV SAPEAAMXDI GGDRFAVDVF DVPGAYEIPL 60
HARTLAEETGR YGAVLGTAFF VXGGIYXHEF VASAVIXGMM NVQLXTGPV LSAVLTPHXY 120
XXSXAXTLLF LALFAVKGME AARACVEILA AREKIAA 157

SEQ ID NO: 41      moltype = AA length = 159
FEATURE          Location/Qualifiers
REGION           1..159
VARIANT          note = T32-28A
                 1
VARIANT          note = optionally absent
source           1..159
                 mol_type = protein
                 organism = synthetic construct

SEQUENCE: 41
MGEVPIGDPK ELNGMELIAAV YLQPIEMEPR GIDLAASLAD IHLEADIHAL KNPNNGFPEG 60
FWMPYLTIAV ALANADTGAI KTGTLMPPMVA DDGPHYGANI AMEKDKKGFF GVGTYALTFL 120
ISNPEKQGFG RHVDEETGVG KWFEPPVVTV FFKYTGTPK 159

SEQ ID NO: 42      moltype = AA length = 184
FEATURE          Location/Qualifiers
REGION           1..184
VARIANT          note = T32-28B
                 1
VARIANT          note = optionally absent
source           1..184
                 mol_type = protein
                 organism = synthetic construct

SEQUENCE: 42
MSQAIGILEL TSIAKGMELG DAMLSANVD LLVSKTISPG KFLLMLGGDI GAIQQAIETG 60
TSQAGEMLVD SLVLANIHPG VLPAISGLNS VDKRQAVGIV ETWSVAACIS AADLAVKGSN 120
VTLVRVHMAF GIGGKCYMVV AGDVLDVAAA VATASLAAGA KGLLVYASII PRPHEAMWRQ 180
MVEG                         184

SEQ ID NO: 43      moltype = AA length = 103
FEATURE          Location/Qualifiers
REGION           1..103
VARIANT          note = T33-09A
                 1
VARIANT          note = optionally absent
source           1..103
                 mol_type = protein
                 organism = synthetic construct

SEQUENCE: 43
MEEVVLITVP SALVAKIAH ALVEERLAAC VNIVPGLTSI YRWQGSVVSD HELLLLKVTT 60
THAFPKLKER VKALHPYTVF EIVALPIAEG NREYLDWLRE NTG 103

SEQ ID NO: 44      moltype = AA length = 122
FEATURE          Location/Qualifiers
REGION           1..122

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VARIANT          note = T33-09B
source           1
                  note = optionally absent
1..122
                  mol_type = protein
                  organism = synthetic construct
SEQUENCE: 44
MVRGIRGAIT VEEDTPAAIL AATIELLLKM LEANGIQSYE ELAAVIFTVT EDLTSAPPAE 60
AARLIGMHRV PLLSAREVPV PGSLPRVIRV LALWNTDTPQ DRVRHVYLNE AVRRLRPDLES 120
AQ                           122

SEQ ID NO: 45      moltype = AA length = 177
FEATURE          Location/Qualifiers
REGION           1..177
VARIANT          note = T33-15A
1
source           note = optionally absent
1..177
mol_type = protein
organism = synthetic construct
SEQUENCE: 45
MSKAKIGIVT VSDRASAGIT ADISGKAIIL ALNLYLTSEW EPIYQVIPDE QDVIEETLIK 60
MADEQDCCLI VTTGGTGPAK RDVTPEATEA VCDRMMPGFG ELMRAESLKE VPTAILSRT 120
AGLRGDSLIV NLPGDPASIS DCLLAVFPAl PYCIDLMEGP YLECNEAMIK PFRPKAK    177

SEQ ID NO: 46      moltype = AA length = 122
FEATURE          Location/Qualifiers
REGION           1..122
VARIANT          note = T33-15B
1
source           note = optionally absent
1..122
mol_type = protein
organism = synthetic construct
SEQUENCE: 46
MVRGIRGAIT VNSDPTSII IATILLLEKM LEANGIQSYE ELAAVIFTVT EDLTSAPPAE 60
AARQIGMHRV PLLSAREVPV PGSLPRVIRV LALWNTDTPQ DRVRHVYLSE AVRRLRPDLES 120
AQ                           122

SEQ ID NO: 47      moltype = AA length = 172
FEATURE          Location/Qualifiers
REGION           1..172
VARIANT          note = T33-21A
1
source           note = optionally absent
1..172
mol_type = protein
organism = synthetic construct
SEQUENCE: 47
MRITTKVGDK GSTRLFGGEE VWKD SPIIEA NGTLDELSF IGEAKHYVDE EMKGILEEIQ 60
NDIYKIMGEI GSKGKLEGIS EERIAWLKL ILRYMEMVN KSFVLPGTl ESAKLDVCRT 120
IARRALRKVL TVTREFGIGA EAAAYLLALS DLLFLALARVI EIEKNKLKEV RS        172

SEQ ID NO: 48      moltype = AA length = 123
FEATURE          Location/Qualifiers
REGION           1..123
VARIANT          note = T33-21B
1
source           note = optionally absent
1..123
mol_type = protein
organism = synthetic construct
SEQUENCE: 48
MPHLVIEATA NLRLETSPGE LLEQANKALF ASQGFGEADI KSRFVITLEAY RQGTAVERA 60
YLHACLSILD GRDIATRTL GASLCAVLAE AVAGGEEGV QVSVEVREME RLSYAKRVVA 120
RQR                           123

SEQ ID NO: 49      moltype = AA length = 158
FEATURE          Location/Qualifiers
REGION           1..158
VARIANT          note = T33-28A
1
source           note = optionally absent
1..158
mol_type = protein
organism = synthetic construct

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SEQUENCE: 49
MSNVNTSFLS PSLVTIRDFD NGQFAVLIG RTGFPADKGD IDLCLDKMIG VRAAQIFLGD 60
DTEDGFKGPH IRIRCVDIDD KHTYNAMVYV DLIVGTGASE VERETAAEEA KLALRVALQV 120
DIADEHSCVT QFEMKLREEL LSSDSFHDPK DEYYKDFL 158

SEQ ID NO: 50      moltype = AA  length = 113
FEATURE
REGION           Location/Qualifiers
1..113
note = T33-28B
VARIANT          1
note = optionally absent
source            1..113
mol_type = protein
organism = synthetic construct

SEQUENCE: 50
MPVIQTFVST PLDHHKRLLL AIIYRIVTRV VLGKPEDLVM MTFHDSTPMH FFGSTDPMVAC 60
VRVEALGGYG PSEPEKVTSI VTAAITAVCG IVADRIFVLY FSPLHCGWNG TNF 113

SEQ ID NO: 51      moltype = AA  length = 103
FEATURE
REGION           Location/Qualifiers
1..103
note = T33-31A
VARIANT          1
note = optionally absent
source            1..103
mol_type = protein
organism = synthetic construct

SEQUENCE: 51
MEEVVLITVP SALVAVKIAH ALVEERLAAC VNIVPGLTSI YREEGSVVSD HELLLLVKTT 60
TDAFPKLKER VKELHPYEV P EIVALPIAEG NREYLDWLRE NTG 103

SEQ ID NO: 52      moltype = AA  length = 7
FEATURE
REGION           Location/Qualifiers
1..7
note = Synthetic peptide
source            1..7
mol_type = protein
organism = synthetic construct

SEQUENCE: 52
QNITEEF 7

SEQ ID NO: 53      moltype = AA  length = 513
FEATURE
REGION           Location/Qualifiers
1..513
note = DS-Cav1
VARIANT          1..25
note = optionally absent
VARIANT          508..513
note = optionally absent
source            1..513
mol_type = protein
organism = synthetic construct

SEQUENCE: 53
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDAKVKLIQ QELDDKYKNAV TELQLLMQST PATNNNRARRE LPRFMNYTLN 120
NAKKTNVNTLS KKRKRRFLGF LLGVGSAIAS GVAVCKVLHL EGEVNIKIKSA LLSTNKAVVS 180
LSNGVSVLTF KVLDLKNYID KQLLPILNKQ SCSISNIEVT IEFQQKNRNL LEITREFSVN 240
AGVTPPVSTY MLTNSELLSL INDMPITNDQ KKLMNSNNVQI VRQQSYSIMC IIKEEVLAYV 300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRTDRG WYCDNAGSVS FFPQAETCKV 360
QSNRNVFCDTM NSLTLPSEVN LCNVNFNPK YDCKIMTSKT DVSSSVITSL GAIIVSCYGKT 420
KCTASNKNRG IIKTFSNGCD YVSNKGVDTV SGNTLYYVN KQEGKSLYVK GEPIINFYDP 480
LVFPSDEFDA SISQVNEKIN QSLAFIRKSD ELL 513

SEQ ID NO: 54      moltype = AA  length = 27
FEATURE
REGION           Location/Qualifiers
1..27
note = T4 fibrin foldon domain
source            1..27
mol_type = protein
organism = synthetic construct

SEQUENCE: 54
GYIPEAPRDG QAYVRKDGEW VLLSTFL 27

SEQ ID NO: 55      moltype = AA  length = 15
FEATURE
REGION           Location/Qualifiers
1..15

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source          note = Linker
               1..15
               mol_type = protein
               organism = synthetic construct
SEQUENCE: 55
GSGGSGSGSG GSGSG                                15

SEQ ID NO: 56          moltype = AA  length = 8
FEATURE          Location/Qualifiers
REGION           1..8
note = Linker
source          1..8
               mol_type = protein
               organism = synthetic construct
SEQUENCE: 56
GGSGGSGS                                8

SEQ ID NO: 57          moltype = AA  length = 8
FEATURE          Location/Qualifiers
REGION           1..8
note = Linker
source          1..8
               mol_type = protein
               organism = synthetic construct
SEQUENCE: 57
GSGGSGSG                                8

SEQ ID NO: 58          moltype = AA  length = 11
FEATURE          Location/Qualifiers
REGION           1..11
note = Helical extension
source          1..11
               mol_type = protein
               organism = synthetic construct
SEQUENCE: 58
EKAAKAEEAA R                                11

SEQ ID NO: 59          moltype = AA  length = 25
FEATURE          Location/Qualifiers
REGION           1..25
note = Signal peptide
source          1..25
               mol_type = protein
               organism = synthetic construct
SEQUENCE: 59
MELLILKANA ITTILTAVTF CFASG                                25

SEQ ID NO: 60          moltype = AA  length = 540
FEATURE          Location/Qualifiers
REGION           1..540
note = DS-Cav1-foldon
VARIANT          1..25
note = optionally absent
source          1..540
               mol_type = protein
               organism = synthetic construct
SEQUENCE: 60
MELLILKANA ITTILTAVTF CFASQONITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDAKVKLIF QELDKYKNAV TELQLLMQST PATNNRARE LPRFMNYTLN 120
NAKKTNVTLS KKRKRRFLGF LLGVGSAIAS GVAVCKVLHL KQLLPILNQ SCISINIEVT 180
LSNGTVNLTF KVLDLKNYID KQLLPILNQ SCISINIEVT IEFQQKNRNL LEITREFSVN 240
AGVTPVSTY MLTNSELLSL INDMPITNDQ KKLMNSNNVQI VRQQSYSIMC IIKEEVLAYV 300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRTDRG WYCDNAGSVS FFPQAETCKV 360
QSNRVFCDTM NSLTLFSEVN LCNVDFNPK YDCKIMTSKT DVSSSVITSL GAIVSCYGKT 420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDT SVGNTLYYVN KQEGKSLYVK GEPIINFYDP 480
LVPPSDEFDA SISQVNEKIN QSLAFIRKSD ELLGYIPEAP RDGQAYVRKD GEWVLLSTFL 540

SEQ ID NO: 61          moltype = AA  length = 474
FEATURE          Location/Qualifiers
REGION           1..474
note = sc9-10 DS-Cav1 A149C Y458C
VARIANT          1..25
note = optionally absent
VARIANT          468..474
note = optionally absent
source          1..474

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mol_type = protein
organism = synthetic construct

SEQUENCE: 61
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDAVKLIK QELDKYKNAV TELQLLMQST PATGSGSAIC SGVAVCKVLH 120
LEGEVNVNIKS ALLSTNKAVV SLSNGVSILT FKVLDLKNYI DKQLLPILNK QSCSISNIET 180
VIEFQQKNNR LLEITREFSV NAGVTPVST YMLTNSELLS LINDMPITND QKKLMSNNVQ 240
IVRQQSYSIM CIIKEEVLAY VVQLPLYGV1 DTPCWLHLTS PLCTTNTKEG SNICLRTD 300
GWYCDNAGSV SFFPQAETCK VQSNRVFCDT MNSRTLPEV NLCNVDIIFNP KYDCKIMTSK 360
TDVSSSVITS LGAIIVSCYGE TKCTASNKNR GIIKTFNSNGC DYVSNKGVDT VSVGNTLYCV 420
NKGEGKSLYV KGEPIINFYD PLVPPSDEFD ASISQVNKEI NQSLAFIRKS DELL 474

SEQ ID NO: 62      moltype = AA length = 474
FEATURE           Location/Qualifiers
REGION            1..474
note = sc9-10 DS-Cav1 A149C Y458C S46G K465Q S215P E92D
VARIANT           1..25
note = optionally absent
VARIANT           468..474
note = optionally absent
source            1..474
mol_type = protein
organism = synthetic construct

SEQUENCE: 62
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLGALRT GWYTSVITIE 60
LSNIKENKCN GTDAVKLIK QELDKYKNAV TDLQLLMQST PATGSGSAIC SGVAVCKVLH 120
LEGEVNVNIKS ALLSTNKAVV SLSNGVSILT FKVLDLKNYI DKQLLPILNK QSCSIPNIET 180
VIEFQQKNNR LLEITREFSV NAGVTPVST YMLTNSELLS LINDMPITND QKKLMSNNVQ 240
IVRQQSYSIM CIIKEEVLAY VVQLPLYGV1 DTPCWLHLTS PLCTTNTKEG SNICLRTD 300
GWYCDNAGSV SFFPQAETCK VQSNRVFCDT MNSRTLPEV NLCNVDIIFNP KYDCKIMTSK 360
TDVSSSVITS LGAIIVSCYGE TKCTASNKNR GIIKTFNSNGC DYVSNKGVDT VSVGNTLYCV 420
NKGEGQSLYV KGEPIINFYD PLVPPSDEFD ASISQVNKEI NQSLAFIRKS DELL 474

SEQ ID NO: 63      moltype = AA length = 522
FEATURE           Location/Qualifiers
REGION            1..522
note = SC-DM (N67I, S215P)
VARIANT           1..25
note = optionally absent
VARIANT           486..522
note = optionally absent
source            1..522
mol_type = protein
organism = synthetic construct

SEQUENCE: 63
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKKIKCN GTDAKIKLIK QELDKYKNAV TELQLLMQST PATNNQARGS GSGRSLGFL 120
GVGSIAISGV AVSKVLHLEG EVNIKIKSALL STNKAVVSL S NGVSVLTSKV LDLKNYIDKQ 180
LLPIVNVNQSC SIPNIEVTIV FQQKNNRRLLE I TREFSVNA VTTPVSTYML TNSELLSLIN 240
DMPITNDQKK LMSNNVQIVR QQSYSIMSII KEEVLAYVQQ LPLYGVIDTP CWKLHTSP 300
TTNTKEGSNI CLTRTDRGWY CDNAGSVSFF PQAETCKVQ S NRVFCDTMNS LTLPSEVNLC 360
NVDIFNPKYD CKIMTSKTDV SSSVITSLGA IVSCYGKTKC TASNKNRGII KTFNSNGCDYV 420
SNKGVDTSV GNTLYYVVKQ EGKSLYVKG P II INFYDPLV FPSDEFDASI SQVNEKINQS 480
LAIFIRKSDEL LSAIGGYIPE APRDGQAYVR KDGEWVLLST FL 522

SEQ ID NO: 64      moltype = AA length = 522
FEATURE           Location/Qualifiers
REGION            1..522
note = SC-TM (N67I, S215P, and E487Q)
VARIANT           1..25
note = optionally absent
VARIANT           486..522
note = optionally absent
source            1..522
mol_type = protein
organism = synthetic construct

SEQUENCE: 64
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKKIKCN GTDAKIKLIK QELDKYKNAV TELQLLMQST PATNNQARGS GSGRSLGFL 120
GVGSIAISGV AVSKVLHLEG EVNIKIKSALL STNKAVVSL S NGVSVLTSKV LDLKNYIDKQ 180
LLPIVNVNQSC SIPNIEVTIV FQQKNNRRLLE I TREFSVNA VTTPVSTYML TNSELLSLIN 240
DMPITNDQKK LMSNNVQIVR QQSYSIMSII KEEVLAYVQQ LPLYGVIDTP CWKLHTSP 300
TTNTKEGSNI CLTRTDRGWY CDNAGSVSFF PQAETCKVQ S NRVFCDTMNS LTLPSEVNLC 360
NVDIFNPKYD CKIMTSKTDV SSSVITSLGA IVSCYGKTKC TASNKNRGII KTFNSNGCDYV 420
SNKGVDTSV GNTLYYVVKQ EGKSLYVKG P II INFYDPLV FPSDEFDASI SQVNEKINQS 480
LAIFIRKSDEL LSAIGGYIPE APRDGQAYVR KDGEWVLLST FL 522

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SEQ ID NO: 65      moltype = AA length = 490
FEATURE          Location/Qualifiers
REGION           1..490
note = HMPV F protein, strain CAN97-83 (A2)
VARIANT          1..18
note = optionally absent
source           1..490
mol_type = protein
organism = synthetic construct
SEQUENCE: 65
MSWKVVIIFSLLITPQHGLK ESYLEESCST ITEGYLSVLR TGWYTNVFTL EVGDVENLTC 60
SDGSPSLIKTE LDLTKSALRE LKTVSADQLA REEQIENPRQ SRFVLGAIAL GVATAAAVTA 120
GVAIAKTIRESSEVTAIKNA LKTTNEAVST LGNGVRVLAF AVRELKDFVS KNLTRAINKN 180
KCDIDDLKMA VSFSQFNRRF LNVVRQFSDN AGITPAISLD LMTDAELARA VSNMPTSAGQ 240
IJKLMLENRAM VRKGPGILL GVGGSIVYM VOLPIFGVID TPCWIVKAAP SCSGKKGNYA 300
CLLRDQGWY CQNAGSTVYY PNEKDCTRG DHVFCDTAACG INVAEQSKEC NINISTTNPY 360
CKVSTGRHPI SMVALSPLGA LVACYKGVSC SIGSNRVGII KQLNKGCSYI TNQDADTVTI 420
DNTVYQLSKV EGEQHVIKGR PVSSSFDPK FPEDQFNVAL DQVFENIENS QALVDQSNRI 480
LSSAEKGNTG          490

SEQ ID NO: 66      moltype = AA length = 490
FEATURE          Location/Qualifiers
REGION           1..490
note = HMPV with A113C, A339C, T160F, I177L
VARIANT          1..18
note = optionally absent
source           1..490
mol_type = protein
organism = synthetic construct
SEQUENCE: 66
MSWKVVIIFSLLITPQHGLK ESYLEESCST ITEGYLSVLR TGWYTNVFTL EVGDVENLTC 60
SDGSPSLIKTE LDLTKSALRE LKTVSADQLA REEQIENPRQ SRFVLGAIAL GVCTAAVTA 120
GVAIAKTIRESSEVTAIKNA LKTTNEAVST LGNGVRVLAF AVRELKDFVS KNLTRALNKN 180
KCDIDDLKMA VSFSQFNRRF LNVVRQFSDN AGITPAISLD LMTDAELARA VSNMPTSAGQ 240
IJKLMLENRAM VRKGPGILL GVGGSIVYM VOLPIFGVID TPCWIVKAAP SCSGKKGNYA 300
CLLRDQGWY CQNAGSTVYY PNEKDCTRG DHVFCDTACG INVAEQSKEC NINISTTNPY 360
CKVSTGRHPI SMVALSPLGA LVACYKGVSC SIGSNRVGII KQLNKGCSYI TNQDADTVTI 420
DNTVYQLSKV EGEQHVIKGR PVSSSFDPK FPEDQFNVAL DQVFENIENS QALVDQSNRI 480
LSSAEKGNTG          490

SEQ ID NO: 67      moltype = AA length = 490
FEATURE          Location/Qualifiers
REGION           1..490
note = HMPV- F with A113C, A120C, A339C, T160F, I177L, and
                Q426C
VARIANT          1..18
note = optionally absent
source           1..490
mol_type = protein
organism = synthetic construct
SEQUENCE: 67
MSWKVVIIFSLLITPQHGLK ESYLEESCST ITEGYLSVLR TGWYTNVFTL EVGDVENLTC 60
SDGSPSLIKTE LDLTKSALRE LKTVSADQLA REEQIENPRQ SRFVLGAIAL GVCTAAVTC 120
GVAIAKTIRESSEVTAIKNA LKTTNEAVST LGNGVRVLAF AVRELKDFVS KNLTRALNKN 180
KCDIDDLKMA VSFSQFNRRF LNVVRQFSDN AGITPAISLD LMTDAELARA VSNMPTSAGQ 240
IJKLMLENRAM VRKGPGILL GVGGSIVYM VOLPIFGVID TPCWIVKAAP SCSGKKGNYA 300
CLLRDQGWY CQNAGSTVYY PNEKDCTRG DHVFCDTACG INVAEQSKEC NINISTTNPY 360
CKVSTGRHPI SMVALSPLGA LVACYKGVSC SIGSNRVGII KQLNKGCSYI TNQDADTVTI 420
DNTVYCLSKV EGEQHVIKGR PVSSSFDPK FPEDQFNVAL DQVFENIENS QALVDQSNRI 480
LSSAEKGNTG          490

SEQ ID NO: 68      moltype = AA length = 542
FEATURE          Location/Qualifiers
REGION           1..542
note = 115-BV (A185P)
VARIANT          1..18
note = optionally absent
VARIANT          490..542
note = optionally absent
source           1..542
mol_type = protein
organism = synthetic construct
SEQUENCE: 68
MSWKVVIIFSLLITPQHGLK ESYLEESCST ITEGYLSVLR TGWYTNVFTL EVGDVENLTC 60
ADGSPSLIKTE LDLTKSALRE LRTVSADQLA REEQIENPRR RRFVLGAIAL GVATAAAVTA 120

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GVAIAKTIRL	ESEVTAIKNA	LKKTNEAVST	LGNGVRVLAT	AVRELKDFVS	KNLTRAINKN	180
KCDIPDLKMA	VFSFSQFNRRF	LNVNRQFSND	AGITPAISLD	LMTDAELARA	VSNMPTSAGQ	240
IKLMLENRAM	VRRKGFGILI	GVGSSVIYM	VQLPIFGVID	TPCWIVKAAP	SCSEKKGNYA	300
CLLRREDQGWY	CQNAGSTVYY	PNEKDCETRG	DHVFCDTAAG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPLGA	LVACYKGVSC	SIGSNRVGII	KQLNKGSYI	TNQDADTVTI	420
DNTVYQLSKV	EGEQHVKGR	PVSSSFDPVK	FPEPDQFNVAL	DQVFESIENS	QALVDQSNRI	480
LSSAEKGNTS	GRENLYFQGG	GGSGYIPEAP	RDGQAYVRKD	GEWVLLSTFL	GGIEGRHHHH	540
HH						542

SEQ ID NO: 69	moltype = AA	length = 646				
FEATURE	Location/Qualifiers					
REGION	1..646					
VARIANT	note = DS-Cav1-foldon-T33-31A					
1..26						
source	note = optionally absent					
1..646						
	mol_type = protein					
	organism = synthetic construct					
SEQUENCE: 69						
MELLILKANV	IATILTAVTF	CFASSQNITE	EFYQSTCSAV	SKGYLSALRT	GWYTSVITIE	60
LSNIKENKCN	GTDALKVLIK	QELDKYKNAV	TELQLLMQST	PATNNRARRE	LPRFMNYTLN	120
NAKKTNVTLS	KKRKRRFLGF	LLGVGSAIAS	GVAVCKVLHL	EGERVNKIKSA	LLSTNKAVVS	180
LSNGVSVLTF	KVLDLKNYID	KQLLPILNKQ	SCSISNIETV	IEFQQKNRNL	LEITREFSVN	240
AGVTPVSTY	MLTNSELLSL	INDMPITNDQ	KKLMNSNNVQI	VRQQSYSIMC	IIKEEVLAYV	300
VQLPLYGVID	TPCWLHHTSP	LCTTNTKEGS	NICLTRTDRG	WYCDNAGSVS	FFPQAETCKV	360
QSNRVFCDTM	NSLTLPSEVN	LCNVDIFNPK	YDCKIMTSKT	DVSSSVITSL	GAIVSCYGKT	420
KCTASNKNRG	IIKTFSNGCD	YVSNKGVDTV	SVGNTLYYVN	KQEGKSLYVK	GEPIINFYDP	480
LVPPSDEFDA	SISQVNEKIN	QSLAFIRKSD	ELLGYIPEAP	RDGQAYVRKD	GEWVLLSTFL	540
GGSMEEVLLI	TVPSALVALV	IAHALVEERL	AACVNIVPGL	TSIYREEGGSV	VSDHELLLV	600
KTTTDAPPKL	KERVKELHPY	EVPEIVALPI	AEGNREYLDW	LRENTG		646

SEQ ID NO: 70	moltype = AA	length = 619				
FEATURE	Location/Qualifiers					
REGION	1..619					
VARIANT	note = DS-Cav1-T33-31A					
1..25						
source	note = optionally absent					
1..619						
	mol_type = protein					
	organism = synthetic construct					
SEQUENCE: 70						
MELLILKANV	IATILTAVTF	CFASSQNITE	EFYQSTCSAV	SKGYLSALRT	GWYTSVITIE	60
LSNIKENKCN	GTDALKVLIK	QELDKYKNAV	TELQLLMQST	PATNNRARRE	LPRFMNYTLN	120
NAKKTNVTLS	KKRKRRFLGF	LLGVGSAIAS	GVAVCKVLHL	EGERVNKIKSA	LLSTNKAVVS	180
LSNGVSVLTF	KVLDLKNYID	KQLLPILNKQ	SCSISNIETV	IEFQQKNRNL	LEITREFSVN	240
AGVTPVSTY	MLTNSELLSL	INDMPITNDQ	KKLMNSNNVQI	VRQQSYSIMC	IIKEEVLAYV	300
VQLPLYGVID	TPCWLHHTSP	LCTTNTKEGS	NICLTRTDRG	WYCDNAGSVS	FFPQAETCKV	360
QSNRVFCDTM	NSLTLPSEVN	LCNVDIFNPK	YDCKIMTSKT	DVSSSVITSL	GAIVSCYGKT	420
KCTASNKNRG	IIKTFSNGCD	YVSNKGVDTV	SVGNTLYYVN	KQEGKSLYVK	GEPIINFYDP	480
LVPPSDEFDA	SISQVNEKIN	QSLAFIRKSD	ELLGMSMEEV	VLITVPSALV	AVKIAHALVE	540
ERLAAACVNIV	PGLTSIYREE	GSVVSDEHELL	LLVKTDTDAF	PKLKERVKEL	H PYEVPEIVA	600
LPIAEGNREY	LDWLRENTG					619

SEQ ID NO: 71	moltype = AA	length = 665
FEATURE	Location/Qualifiers	
REGION	1..665	
VARIANT	note = DS-Cav1-foldon-T33-15B	
1..25		
source	note = optionally absent	
1..665		
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 71		

MELLILKANV	IATILTAVTF	CFASSQNITE	EFYQSTCSAV	SKGYLSALRT	GWYTSVITIE	60
LSNIKENKCN	GTDALKVLIK	QELDKYKNAV	TELQLLMQST	PATNNRARRE	LPRFMNYTLN	120
NAKKTNVTLS	KKRKRRFLGF	LLGVGSAIAS	GVAVCKVLHL	EGERVNKIKSA	LLSTNKAVVS	180
LSNGVSVLTF	KVLDLKNYID	KQLLPILNKQ	SCSISNIETV	IEFQQKNRNL	LEITREFSVN	240
AGVTPVSTY	MLTNSELLSL	INDMPITNDQ	KKLMNSNNVQI	VRQQSYSIMC	IIKEEVLAYV	300
VQLPLYGVID	TPCWLHHTSP	LCTTNTKEGS	NICLTRTDRG	WYCDNAGSVS	FFPQAETCKV	360
QSNRVFCDTM	NSLTLPSEVN	LCNVDIFNPK	YDCKIMTSKT	DVSSSVITSL	GAIVSCYGKT	420
KCTASNKNRG	IIKTFSNGCD	YVSNKGVDTV	SVGNTLYYVN	KQEGKSLYVK	GEPIINFYDP	480
LVPPSDEFDA	SISQVNEKIN	QSLAFIRKSD	ELLGYIPEAP	RDGQAYVRKD	GEWVLLSTFL	540
GGSMVRCIGR	AITVNSDTPT	SIIIAITLLL	EKMLEANGIQ	SYEELAAVIF	TVTEDLTSAF	600
PAEAARQIGM	HRVPLLSARE	VPVPGSLPRV	IRVLALWNTD	TPQDRVRHVY	LSEAVRLRPD	660
LESAQ						665

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SEQ ID NO: 72      moltype = AA length = 638
FEATURE          Location/Qualifiers
REGION           1..638
note = DS-Cav1-T33-15B
VARIANT          1..25
note = optionally absent
source            1..638
mol_type = protein
organism = synthetic construct

SEQUENCE: 72
MELLILKANV IATILTAVTF CFASSQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDALKVLIK QELDKYKNAV TELQLLMQST PATNNRARRR LPRFMNYTLN 120
NAKKTNTTLS KKRKRRFLGF LLGVGSAIAS GVAVCKVLHL EGEVNIKISA LLSTNKAVVS 180
LSNGVSVLTF KVLDLKNYID KQLLPILNKQ SCSISNIETV IEFQQKNNRLEITREFSVN 240
AGVTTPVSTY MLTNSELLSL INDMPITNQ KKLMSNNVQI VRQQSYSIMC IIKEEVLAYV 300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLRTDRG WYCDNAGSVS FFPQAETCKV 360
QSNRVRFCDTM NSLTLPEVN LCNVDIFNPK YDCKIMTSKT DVSSSVITSL GAIIVSCYGKT 420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDTV SVGNTLYYVNV KQEGKSLYVK GEPIINFYDP 480
LVFPPSDEFDA SISQVNEKIN QSLAFIRKSD ELLGGSMVRG IRGAITVNSD TPTSIITI 540
LILLEKMLEAN GIQSYPELAA VIFTVTEDLT SAFFPAEARQ IGMHRVPLLS AREVPVPGSL 600
PRVIRVLALW NTDTPQDRVR HVYLSEAVRL RPDLESQA 638

SEQ ID NO: 73      moltype = AA length = 769
FEATURE          Location/Qualifiers
REGION           1..769
note = DS-Cav1-foldon-I53-50A
VARIANT          1..25
note = optionally absent
source            1..769
mol_type = protein
organism = synthetic construct

SEQUENCE: 73
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDALKVLIK QELDKYKNAV TELQLLMQST PATNNRARRR LPRFMNYTLN 120
NAKKTNTTLS KKRKRRFLGF LLGVGSAIAS GVAVCKVLHL EGEVNIKISA LLSTNKAVVS 180
LSNGVSVLTF KVLDLKNYID KQLLPILNKQ SCSISNIETV IEFQQKNNRLEITREFSVN 240
AGVTTPVSTY MLTNSELLSL INDMPITNQ KKLMSNNVQI VRQQSYSIMC IIKEEVLAYV 300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLRTDRG WYCDNAGSVS FFPQAETCKV 360
QSNRVRFCDTM NSLTLPEVN LCNVDIFNPK YDCKIMTSKT DVSSSVITSL GAIIVSCYGKT 420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDTV SVGNTLYYVNV KQEGKSLYVK GEPIINFYDP 480
LVFPPSDEFDA SISQVNEKIN QSLAFIRGYI PEAPRDQAY VRKDGEWVLL STFLGSGSHH 540
HHHHHHGGSG GSGSEKAAKA EEAARKMEE FKHKHIVAVL RANSVEEAI KAVAVFAGGV 600
HIIIEITPTV DADTVIKALS VLKEKGAIIG AGTVTSVEQC RKAIVESGAEF IVSPHLDEEI 660
SQFCKEKGVF YMPGVMTPTV LVKAMKLGHT ILKLFPGEVGV GPQFVKAMKG PFPNVKFVPT 720
GGVNLDNVCE WFKAGVLAVG VGSALVKGTP DEVREKAKAF VEKIRGCTE 769

SEQ ID NO: 74      moltype = AA length = 730
FEATURE          Location/Qualifiers
REGION           1..730
note = DS-Cav1-I53-50A
VARIANT          1..25
note = optionally absent
source            1..730
mol_type = protein
organism = synthetic construct

SEQUENCE: 74
MELLILKANV IATILTAVTF CFASSQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDALKVLIK QELDKYKNAV TELQLLMQST PATNNRARRR LPRFMNYTLN 120
NAKKTNTTLS KKRKRRFLGF LLGVGSAIAS GVAVCKVLHL EGEVNIKISA LLSTNKAVVS 180
LSNGVSVLTF KVLDLKNYID KQLLPILNKQ SCSISNIETV IEFQQKNNRLEITREFSVN 240
AGVTTPVSTY MLTNSELLSL INDMPITNQ KKLMSNNVQI VRQQSYSIMC IIKEEVLAYV 300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLRTDRG WYCDNAGSVS FFPQAETCKV 360
QSNRVRFCDTM NSLTLPEVN LCNVDIFNPK YDCKIMTSKT DVSSSVITSL GAIIVSCYGKT 420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDTV SVGNTLYYVNV KQEGKSLYVK GEPIINFYDP 480
LVFPPSDEFDA SISQVNEKIN QSLAFIRGGS GSGSEKAAKA EEAARKMEE LFKKHIVAV 540
LRANSVEEAI EKAVAVFAGG VHLIEITFTV PDADTVIKAL PDAKKEGAI GAGTVTSVEQ 600
CRKAIVESGAE FIVSPHLDEE ISQFCKEKGV FYMPGVMTPTV ELVKAMKLGHT TILKLFPGEV 660
VGQFVKAMK GPFPNVKFVPT TGGVNLDNVCE EWFKAGVLAV GVGSAVKGT PDEVREKAKA 720
FVEKIRGCTE 730

SEQ ID NO: 75      moltype = AA length = 676
FEATURE          Location/Qualifiers
REGION           1..676
note = DS-Cav1-I32-28A
VARIANT          1..25
note = optionally absent

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source          1..676
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 75
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDAVKLIK QELDKYKNAV TELQLLMQST PATNNRARRRE LPRFMNYTLN 120
NAKKTNVTLS KKRKRRFLGF LLGVGSIAAS GVAVCKVLHL EGEVNKIKSA LLSTNKAVVS 180
LSNGSVSLTF KVLDLKNYID KQLLPILNKQ SCSIISNIETV IEFQQKNRLE LEITREFSVN 240
AGVTPVSTY MLTNSELLSL INDMPITNDQ KKLMSNNVQI VRQQSYSIMC IIKEEVLAYV 300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRLDRG WYCDNAGSVS FFPQAETCKV 360
QSNRVCDFM NSLTLPEVN LCNVDIFNPK YDCKIMTSKT DVSSSVITSL GAIVSCYGKT 420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDTV SVGNTLYYVN KQEGKSLYVK GEPIINFYDP 480
LVFPSDEFDA SISQVNEKIN QSLAFIRKSD ELLGGSGGSS SDDARIAAIG DVDELNSQIG 540
VILLAEPPLDD VRAALSQIYH DLFDLGGECLC IPGHAAITED HLLRLALWLWV HYNGOLPPLE 600
EFILPGGARG ALAHVCRTV CRRAESRIKA LGASEPLNIA PAAYVNLLSD LLFVLARVLN 660
RAAGGADVLW DRTRAH                                         676

SEQ ID NO: 76          moltype = AA length = 730
FEATURE             Location/Qualifiers
REGION              1..730
                     note = DS-Cav1-8GS-He1Ext-I53-50A (F10)
VARIANT              1..25
                     note = optionally absent
source          1..730
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 76
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDAVKLIK QELDKYKNAV TELQLLMQST PATNNRARRRE LPRFMNYTLN 120
NAKKTNVTLS KKRKRRFLGF LLGVGSIAAS GVAVCKVLHL EGEVNKIKSA LLSTNKAVVS 180
LSNGSVSLTF KVLDLKNYID KQLLPILNKQ SCSIISNIETV IEFQQKNRLE LEITREFSVN 240
AGVTPVSTY MLTNSELLSL INDMPITNDQ KKLMSNNVQI VRQQSYSIMC IIKEEVLAYV 300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRLDRG WYCDNAGSVS FFPQAETCKV 360
QSNRVCDFM NSLTLPEVN LCNVDIFNPK YDCKIMTSKT DVSSSVITSL GAIVSCYGKT 420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDTV SVGNTLYYVN KQEGKSLYVK GEPIINFYDP 480
LVFPSDEFDA SISQVNEKIN QSLAFIRGSG GSGSGEAKA AEEAARKMEE LFKKHHKIVAV 540
LRANSVEEAI EKAVAVFAGG VHLIEITPTV PDADTVIKAL SVLKEKGAAI GAGTVTSVEQ 600
CRKAVESGAE FIVSPHLDEE ISQFCKEKGV FYMPGVMTPT ELVKAMKLGH TILKLFPGEV 660
VGPFQFKAMK GPFPNVKFVP TGGVNLDNV EWFKAGVLAV GVGSALVKGT PDEVREKAKA 720
FVEKIRGCTE                                         730

SEQ ID NO: 77          moltype = AA length = 764
FEATURE             Location/Qualifiers
REGION              1..764
                     note = DS-Cav1-foldon-15GS-He1Ext-I53-50A (F14)
VARIANT              1..25
                     note = optionally absent
source          1..764
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 77
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60
LSNIKENKCN GTDAVKLIK QELDKYKNAV TELQLLMQST PATNNRARRRE LPRFMNYTLN 120
NAKKTNVTLS KKRKRRFLGF LLGVGSIAAS GVAVCKVLHL EGEVNKIKSA LLSTNKAVVS 180
LSNGSVSLTF KVLDLKNYID KQLLPILNKQ SCSIISNIETV IEFQQKNRLE LEITREFSVN 240
AGVTPVSTY MLTNSELLSL INDMPITNDQ KKLMSNNVQI VRQQSYSIMC IIKEEVLAYV 300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRLDRG WYCDNAGSVS FFPQAETCKV 360
QSNRVCDFM NSLTLPEVN LCNVDIFNPK YDCKIMTSKT DVSSSVITSL GAIVSCYGKT 420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDTV SVGNTLYYVN KQEGKSLYVK GEPIINFYDP 480
LVFPSDEFDA SISQVNEKIN QSLAFIRGSG PEAPRDRGQAY VRKDGEWVLL STFLGSGGSG 540
SGSGGSGGSGE KAAKABEAAR KMEELFKKKH IVAVLRANSV EEAIEKAVAV FAGGVHLIEI 600
TFTVPTADTV I KALSVLKEK GAIIGAGTVT SVEQCRKAVE SGAEFIVSPH LDEEISQFCK 660
EKGVFYMPGV MTPTELVKAM KLGHТИЛКЛР PGЕVVGPOFV KAMKGPPNV KFVPTGGVNL 720
DNVCEWFKAG VLAVGVGSAL VKGTPDEVRE KAKAFVEKIR GCTE                                         764

SEQ ID NO: 78          moltype = AA length = 774
FEATURE             Location/Qualifiers
REGION              1..774
                     note = HMPV F wt_CAN97-83 strain-I53-50A
VARIANT              1..18
                     note = optionally absent
source          1..774
               mol_type = protein
               organism = synthetic construct

SEQUENCE: 78
MSWKVVIIFS LLITPQHGLK ESYLEESCST ITEGYLSVLR TGWYTNVFTL EVGDVENLTC 60

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SDGPSLIKTE	LDLTKSALRE	LKTVSADQLA	REEQIENPRQ	SRFVLGAI	GVATAAAVTA	120
GVAIAKTIRL	ESEVTAIKNA	LKTTNEAVST	LGNGVRVLAT	AVRELKDFVS	KNLTRAINKN	180
KCDIDDLKMA	VSFQSQFNRRF	LNVRQFSDN	AGITPAISLD	LMTDAELARA	VSNMPTSAGQ	240
IKLMLLENRAM	VRRKGPGILI	GVYGSVIVM	VQLPIFGVID	TPCWIVKAAP	SCSGKKGNYA	300
CLLRDQGWY	CQNAGSTVYY	PNEKDCTRG	DHFCDTAAG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPGLA	LVACYKGVC	SIGSNRVGII	KQLNKGSYI	TNQDADTVTI	420
DNTVYQLSKV	EGEQHVIKGR	PVSSSFDPIK	FPEDQFNVAL	DQVFENIENS	QALVDQSNRI	480
LSSAEKGNTG	PIIVILLIAV	LGSSMILVSI	FIIKKTKKP	TGAPPELSGV	TNNGFIPHSG	540
SGSHHHHHHH	HGGSGGSGSE	KAAKAEEAAR	KMEELFKKH	IVAVLTRANSV	EEAIEKAVAV	600
FAGGVHLIEI	FTFTVADT	IKALSVLK	GAIIAGAGTV	SVEQCRKAV	SGAEFIVSPH	660
LDEEISQFC	EKGVFYMPGV	MTPTELVKAM	KLGHТИLKL	PGEVVGPFQF	KAMKGPPNV	720
KFVPTGGVNL	DNVCEWFKA	VLAvgVGSA	VKGTPDEVRE	AKAFVEKIR	GCTE	774

SEQ ID NO: 79	moltype = AA length = 725					
FEATURE	Location/Qualifiers					
REGION	1..725					
VARIANT	note = HMPV F A113C_A339C_T160F_I177L-I53-50A 1..18					
source	note = optionally absent 1..725					
SEQUENCE: 79	mol_type = protein organism = synthetic construct					
MSWKVVIIFS	LLITPQHGLK	ESYLEESCST	ITEGYLSVLR	TGWTYTNVFTL	EVGDVENLTC	60
SDGPSLIKTE	LDLTKSALRE	LKTVSADQLA	REEQIENPRQ	SRFVLGAI	GVCTAAVTA	120
GVAIAKTIRL	ESEVTAIKNA	LKTTNEAVST	LGNGVRVLAF	AVRELKDFVS	KNLTRALNKN	180
KCDIDDLKMA	VSFQSQFNRRF	LNVRQFSDN	AGITPAISLD	LMTDAELARA	VSNMPTSAGQ	240
IKLMLLENRAM	VRRKGPGILI	GVYGSVIVM	VQLPIFGVID	TPCWIVKAAP	SCSGKKGNYA	300
CLLRDQGWY	CQNAGSTVYY	PNEKDCTRG	DHFCDTACG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPGLA	LVACYKGVC	SIGSNRVGII	KQLNKGSYI	TNQDADTVTI	420
DNTVYQLSKV	EGEQHVIKGR	PVSSSFDPIK	FPEDQFNVAL	DQVFENIENS	QALVDQSNRI	480
LSSAEKGNTG	GSGSHHHHHH	HGGSGGSGS	EAAKAEEAAR	RKMEELFKKH	KIVAVLTRANS	540
VEEAIEKAVA	VFAGGVHLIE	ITFTVADT	VIKALSVLK	KGAIIGAGTV	TSVEQCRKAV	600
ESGAEFIVSP	HLDEEISQFC	EKGVFYMPGV	MTPTELVKAM	MKLGHТИLKL	FPGEVVGPFQF	660
VKAMKGPPNV	VKFVPTGGVN	LDNVCEWFKA	VLAvgVGSA	LVKGTPDEVRE	EKAKAFVEKI	720
RGCTE						725
SEQ ID NO: 80	moltype = AA length = 725					
FEATURE	Location/Qualifiers					
REGION	1..725					
VARIANT	note = HMPV F A113C_A339C_T160F_I177L_A120C, Q426C - I53-50A 1..18					
source	note = optionally absent 1..725					
SEQUENCE: 80	mol_type = protein organism = synthetic construct					
MSWKVVIIFS	LLITPQHGLK	ESYLEESCST	ITEGYLSVLR	TGWTYTNVFTL	EVGDVENLTC	60
SDGPSLIKTE	LDLTKSALRE	LKTVSADQLA	REEQIENPRQ	SRFVLGAI	GVCTAAVTC	120
GVAIAKTIRL	ESEVTAIKNA	LKTTNEAVST	LGNGVRVLAF	AVRELKDFVS	KNLTRALNKN	180
KCDIDDLKMA	VSFQSQFNRRF	LNVRQFSDN	AGITPAISLD	LMTDAELARA	VSNMPTSAGQ	240
IKLMLLENRAM	VRRKGPGILI	GVYGSVIVM	VQLPIFGVID	TPCWIVKAAP	SCSGKKGNYA	300
CLLRDQGWY	CQNAGSTVYY	PNEKDCTRG	DHFCDTACG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPGLA	LVACYKGVC	SIGSNRVGII	KQLNKGSYI	TNQDADTVTI	420
DNTVYCLSKV	EGEQHVIKGR	PVSSSFDPIK	FPEDQFNVAL	DQVFENIENS	QALVDQSNRI	480
LSSAEKGNTG	GSGSHHHHHH	HGGSGGSGS	EAAKAEEAAR	RKMEELFKKH	KIVAVLTRANS	540
VEEAIEKAVA	VFAGGVHLIE	ITFTVADT	VIKALSVLK	KGAIIGAGTV	TSVEQCRKAV	600
ESGAEFIVSP	HLDEEISQFC	EKGVFYMPGV	MTPTELVKAM	MKLGHТИLKL	FPGEVVGPFQF	660
VKAMKGPPNV	VKFVPTGGVN	LDNVCEWFKA	VLAvgVGSA	LVKGTPDEVRE	EKAKAFVEKI	720
RGCTE						725
SEQ ID NO: 81	moltype = AA length = 703					
FEATURE	Location/Qualifiers					
REGION	1..703					
VARIANT	note = sc-DS2-I53-50A 1..25					
source	note = optionally absent 1..703					
SEQUENCE: 81	mol_type = protein organism = synthetic construct					
MELLILKANA	ITTILTAVTF	CFASGQNITE	EFYQSTCSAV	SKGYLSALRT	GWYTSVITIE	60
LSNIKENKCN	GTDALKLIK	QELDKYKNAV	TELQLLMQST	PATGSGSCIA	SGVAVCKVLH	120
LEGEVNKIKS	ALLSTNKAVV	SLSNGVSVLT	FKVLDLKNYI	DKQLLPILINK	QSCSISNIET	180
VIEFQQKNNR	LLEITREFSV	NAGVTPVST	YMLTNSELLS	LINDMPITND	QKKLMSNNVQ	240
IVRQQSYSIM	CIKEEVLAY	VVQLPLYGV	DTPCWKLHTS	PLCTTNTKEG	SNICLRTDR	300

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GWYCDNAGSV	SFFPQAETCK	VQSNRVCFT	MNSLTLPS	EV NLCNVDFNP	KYDCKIMTSK	360
TDVSSVITS	LGAIVSCYGE	TCKTASNKNR	GIIKTFNSNC	DYVSNKGVDT	VSVGNTLYCV	420
NKQEGKSLYV	KGEPIINFYD	PLVFPSEDFD	ASISQVNNEKI	NQSLAFIRGS	GSHHHHHHHH	480
GSGGGSGSEK	AKAEEAARK	MEELFKHHKI	VAVLTRANSVE	EAIEKAVAVF	AGGVHLIET	540
FTVFDADTVI	KALSVLKEKG	AIIGAGTVTS	VEQCRKAVES	GAEIFIVSPHL	DEEISQFCKE	600
KGVFYMPGVM	PPTELVKAMK	LGHTILKLFP	GEVVGPPQFV	AMKGPFPNVK	FVPTGGVNLD	660
NVCEWFKAGV	LAvgVGsALV	KGTPDEVREK	AKAFVEKIRG	CTE		703

SEQ ID NO: 82	moltype = AA	length = 742				
FEATURE	Location/Qualifiers					
REGION	1..742					
	note = tc-DS2-I53-50A					
VARIANT	1..25					
	note = optionally absent					
source	1..742					
	mol_type = protein					
	organism = synthetic construct					
SEQUENCE: 82						
MELLILKANA	ITTILTAVTF	CFASQGNITE	EFYQSTCSAV	SKGYLSALRT	GWYTSVITIE	60
LSNIKENKCN	GTDALKLIK	QELDKYKNAV	TELQLLMQST	PATNNRARRE	LPRFMNYTLN	120
NAKKTNTVLS	KKRKRRLFLGF	LLGVGSCIAS	GVAVCKVLHL	EGEVNKKSA	LLSTNKAVVS	180
LSNGVSLTF	KVLDLKNYID	KQLLPILNKQ	SCSISNIETV	IEFQQKNRNL	LEITREFSVN	240
AGVTTPVSTY	MLTNSELLSL	INDMPITNDQ	KKLMSSNNVQI	VRQQSYSIMC	IIKEEVLAYV	300
VQLPLYGVID	TPCWKLHTSP	LCTTNTKEGS	NICLTRTDRG	WYCDNAGSVS	FFPQAETCKV	360
QSNRVFCDTM	NSLTLPS	LCNVDFNPK	YDCKIMTSKT	DVSSSVITSL	GAIVSCYGKT	420
KCTASNKRNG	IIKTFNSNGCD	YVSNKGVDTV	SVGNTLYCVN	KQEGKSLYVK	GEPIINFYDP	480
LVPFSDEFDA	SISQVNEKIN	QSLAFIRGS	SHHHHHHHHG	GSGGGSGSEKA	AKAEEAARKM	540
EELFKKKHV	AVLTRANSVE	GGVHLIEITF	TVPDADTVIK	ALSVLKEKGA	600	
IIAGAGTVTSV	EQCRKAVESG	AEFIVSPHL	EEISQFCKE	GVFYMPGVM	PTELVKAMKL	660
GHTILKLFPG	EVVGPPQFV	MKGPFPPNVK	VPTGGVNLDN	VCEWFKAGVL	AVGVGSALVK	720
GTpDEVREKA	KAFVEKIRGC	TE				742

SEQ ID NO: 83	moltype = AA	length = 734				
FEATURE	Location/Qualifiers					
REGION	1..734					
	note = DS-Cav1-12GS-HelExt-I53-50A (F11)					
VARIANT	1..25					
	note = optionally absent					
source	1..734					
	mol_type = protein					
	organism = synthetic construct					
SEQUENCE: 83						
MELLILKANA	ITTILTAVTF	CFASQGNITE	EFYQSTCSAV	SKGYLSALRT	GWYTSVITIE	60
LSNIKENKCN	GTDALKLIK	QELDKYKNAV	TELQLLMQST	PATNNRARRE	LPRFMNYTLN	120
NAKKTNTVLS	KKRKRRLFLGF	LLGVGSAIAS	GVAVCKVLHL	EGEVNKKSA	LLSTNKAVVS	180
LSNGVSLTF	KVLDLKNYID	KQLLPILNKQ	SCSISNIETV	IEFQQKNRNL	LEITREFSVN	240
AGVTTPVSTY	MLTNSELLSL	INDMPITNDQ	KKLMSSNNVQI	VRQQSYSIMC	IIKEEVLAYV	300
VQLPLYGVID	TPCWKLHTSP	LCTTNTKEGS	NICLTRTD RG	WYCDNAGSVS	FFPQAETCKV	360
QSNRVFCDTM	NSLTLPS	LCNVDFNPK	YDCKIMTSKT	DVSSSVITSL	GAIVSCYGKT	420
KCTASNKRNG	IIKTFNSNGCD	YVSNKGVDTV	SVGNTLYYV	KQEGKSLYVK	GEPIINFYDP	480
LVPFSDEFDA	SISQVNEKIN	QSLAFIRGS	GSGGGSGSE	AAKAEFAAR	KMEEFLKKH	540
IVAVLTRANSVE	EEAIEKAVAV	FAGGVHLIEI	TFTVPA DTV	I KALSVLKEK	GAIIGAGTVT	600
SVEQCRKAVSE	SGAEFIVSPH	LDEEISQFC	EKGVFYMPGV	MPTTELVKAM	KLGHТИLKL	660
PGEVVGPQFV	KAMKGPFPNV	KFVPTGGVNL	DNVCEWFKAG	VLAvgVGsAL	VKGTPDEVRE	720
KAKAFVEKIR	CCTE					734

SEQ ID NO: 84	moltype = AA	length = 738
FEATURE	Location/Qualifiers	
REGION	1..738	
	note = DS-Cav1-16GS-HelExt-I53-50A (F12)	
VARIANT	1..25	
	note = optionally absent	
source	1..738	
	mol_type = protein	
	organism = synthetic construct	
SEQUENCE: 84		

MELLILKANA	ITTILTAVTF	CFASQGNITE	E FYQSTCSAV	SKGYLSALRT	GWYTSVITIE	60
LSNIKENKCN	GTDALKLIK	QELDKYKNAV	TELQLLMQST	PATNNRARRE	LPRFMNYTLN	120
NAKKTNTVLS	KKRKRRLFLGF	LLGVGSAIAS	GVAVCKVLHL	EGEVNKKSA	LLSTNKAVVS	180
LSNGVSLTF	KVLDLKNYID	KQLLPILNKQ	SCSISNIETV	IEFQQKNRNL	LEITREFSVN	240
AGVTTPVSTY	MLTNSELLSL	INDMPITNDQ	KKLMSSNNVQI	VRQQSYSIMC	IIKEEVLAYV	300
VQLPLYGVID	TPCWKLHTSP	LCTTNTKEGS	NICLTRTD RG	WYCDNAGSVS	FFPQAETCKV	360
QSNRVFCDTM	NSLTLPS	LCNVDFNPK	YDCKIMTSKT	DVSSSVITSL	GAIVSCYGKT	420
KCTASNKRNG	IIKTFNSNGCD	YVSNKGVDTV	SVGNTLYYV	KQEGKSLYVK	GEPIINFYDP	480
LVPFSDEFDA	SISQVNEKIN	QSLAFIRGS	GSGGGSGSE	SGGEKAAKAE	EAARKMELF	540
KHHKIVAVLR	ANSVEEAIEK	AVAVFAGGVH	LIEITFTV	ADTVIKALSV	LKEKGAIIGA	600

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GTVTSVEQCR KAVESGAEFI VSPHLDEEIS QFCKEKGVFY MPGVMPTTEL VKAMKLGHTI	660
LKLFPGEVVG PQFVKAMKGP FPNVKFVPTG GVNLDNVCEW FKAGVLAVGV GSALVKGTPD	720
EVREKAKAFV EKIRGCTE	738
SEQ ID NO: 85 moltype = AA length = 759	
FEATURE Location/Qualifiers	
REGION 1..759	
VARIANT note = DS-Cav1-foldon-10GS-HeExt-I53-50A (F13)	
1..25	
note = optionally absent	
source 1..759	
mol_type = protein	
organism = synthetic construct	
 SEQUENCE: 85	
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE	60
LSNIKENKCN GTDAVKLIK QELDKYKNAV TELQLLMQST PATNNNRARRE LPRFMNYTLN	120
NAKKTNTVLS KKRKRRFLGF LLGVGSAIAS GVAVKVLHL EGEVNKIKSA LLSTNKAVVS	180
LSNGVSVLTF KVLDLKNYID KQLLPILNKQ SCSISNIETV IEFQQKNRNL LEITREFSVN	240
AGVTPVSTY MLTNSELLSL INDMPITNDQ KKLMSNNVQI VRQQSYSIMC IIKEEVLAYV	300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRTDRG WYCDNAGSVS FFPQAETCKV	360
QSNSRVFCDTM NSLTLPSEVN LCNVDIFNPK YDCKIMTSKT DVSSSVITSL GAIVSCYGKT	420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDTV SVGNTLYYVN KQEGKSLYVK GEPIINFYDP	480
LVFPSPDEFDA SISQVNEKIN QSLAFIRGYI PEAPRDQAY VRKDGEWLL STFLGSGGSG	540
SGSGEKAAKA EEAARKMEEL FKKHKIVAVL RANSVEEAIE KAVAVFAGGV HLIEITFTVP	600
DADTVIKALS VLKEKGAIIG AGTVTSVEQC RKAESGAEF IVSPHLDEEI SQFCKEKGVF	660
YMPGVMTPTE LVKAMKLGHT ILKLFPGEVV GPQFVKAMKG PFPNVKFVPT GGVNLDNVCE	720
WFKAGVLAVG VGSALVKGTP DEVREKAKAF VEKIRGCTE	759
 SEQ ID NO: 86 moltype = AA length = 769	
FEATURE Location/Qualifiers	
REGION 1..769	
VARIANT note = DS-Cav1-foldon-20GS-HeExt-I53-50A (F15)	
1..25	
note = optionally absent	
source 1..769	
mol_type = protein	
organism = synthetic construct	
 SEQUENCE: 86	
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE	60
LSNIKENKCN GTDAVKLIK QELDKYKNAV TELQLLMQST PATNNNRARRE LPRFMNYTLN	120
NAKKTNTVLS KKRKRRFLGF LLGVGSAIAS GVAVKVLHL EGEVNKIKSA LLSTNKAVVS	180
LSNGVSVLTF KVLDLKNYID KQLLPILNKQ SCSISNIETV IEFQQKNRNL LEITREFSVN	240
AGVTPVSTY MLTNSELLSL INDMPITNDQ KKLMSNNVQI VRQQSYSIMC IIKEEVLAYV	300
VQLPLYGVID TPCWKLHTSP LCTTNTKEGS NICLTRTDRG WYCDNAGSVS FFPQAETCKV	360
QSNSRVFCDTM NSLTLPSEVN LCNVDIFNPK YDCKIMTSKT DVSSSVITSL GAIVSCYGKT	420
KCTASNKNRG IIKTFNSNGCD YVSNKGVDTV SVGNTLYYVN KQEGKSLYVK GEPIINFYDP	480
LVFPSPDEFDA SISQVNEKIN QSLAFIRGYI PEAPRDQAY VRKDGEWLL STFLGSGGSG	540
SGSGGSGGG SSGSEKAAKA EEAARKMEEL FKKHKIVAVL RANSVEEAIE KAVAVFAGGV	600
HLIEITFTVP DADTVIKALS VLKEKGAIIG AGTVTSVEQC RKAESGAEF IVSPHLDEEI	660
SQFCKEKGVF YMPGVMTPTE LVKAMKLGHT ILKLFPGEVV GPQFVKAMKG PFPNVKFVPT	720
GGVNLDNVCE WFKAGVLAVG VGSALVKGTP DEVREKAKAF VEKIRGCTE	769
 SEQ ID NO: 87 moltype = AA length = 736	
FEATURE Location/Qualifiers	
REGION 1..736	
VARIANT note = sc9-10 DS-Cav1 A149C Y458C-foldon-I53-50A embodiment	
1..25	
note = optionally absent	
VARIANT 469..474	
note = optionally absent	
source 1..736	
mol_type = protein	
organism = synthetic construct	
 SEQUENCE: 87	
MELLILKANA ITTILTAVTF CFASGQNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE	60
LSNIKENKCN GTDAVKLIK QELDKYKNAV TELQLLMQST PATGSGSAIC SGVAVCKVLH	120
LEGEVNKIKS ALLSTNKAVV SLSNGVSVLT FKVLDDLKYID DKQLLPILNK QSCSISNIET	180
VIEFQQKNRNL LLEITREFSV NAGVTPVSTY YMLTNSSELLS LINDMPITND QKKLMSNNVQ	240
IVRQQSYSIM CIIKEEVLAY VVQLPLYGVID DTPCWKLHTS PLCTTNTKEG SNICLTRTD	300
GWYCDNAGSV SFFPQAETCK VQSNRVCFTD MNSRTLPSFV NLCNVDFNP KYDCKIMTSK	360
TDVSSSVITS LGAIVSCYGK TKCTASNKNR GIICKTFNSNGC DYVSNKGVDT VSVGNTLYCV	420
NKQEGKSLYVK KGEPIINFYD PLVFPSPDEFDA ASISQVNEKIN QSLAFIRKS DELLGYIPEA	480
PRDGQAYVRK DGEWLLSTF LGSGSHHHHH HHHGGSGGG SEKAAKAEEA ARKMEELFKK	540
HKIVAVRLAN SVEEAIEKAV AVFAGGVHLI EITFTVPDAD TVIKALSVLK EKGAIIGAGT	600
VTSVEQCRKA VESGAEFIVS PHLDEEISQF CKEKGVFYIMP GVMTPTELVK AMKLGLHTILK	660
LFPGEVVGPGQ PVKAMKGFP NVKFPVPTGGV NLNDNVCEWFK AGVLAVGVGS ALVKGTPDEV	720

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REKAKAFVEK IRGCTE	736
SEQ ID NO: 88 moltype = AA length = 697 FEATURE Location/Qualifiers REGION 1..697 note = sc9-10 DS-Cav1 A149C Y458C - F10 embodiment VARIANT 1..25 note = optionally absent VARIANT 469..474 note = optionally absent source 1..697 mol_type = protein organism = synthetic construct	
SEQUENCE: 88 MELLILKANA ITTILTAVTF CFASQGNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE 60 LSNIKENKCN GTDAVKLIK QELDKYKNAV TDLQLLMQST PATGSGSAIC SGVAVCKVLH 120 LEGEVNKIKS ALLSTNKAVV SLSNGVSVLT FKVLDLKNYI DKQLLPILNK QSCSISNIET 180 VIEFQQKNRR LLEITREFSV NAGVTPVST YMMLTNSELLS LINDDMPITND QKKLMSNNVQ 240 IVRQQSYSIM CIIKEEVLAY VVQLPLYGVI DTPCWKLHTS PLCTTNTKEG SNICLRTD 300 GWYCDNAGSV SFFPQAETCK VQSNRVCFTD MNSRTLPSVE NLCNVDIIFNP KYDCKIMTSK 360 TDVSSSVITS LGAIIVSCYKG TKCTASNKR GIICKTFSNCG DYVSNKGVDT VSVGNTLYCV 420 NKQEGQSLYV KGEPIINFYD PLVFPDSDEFD ASISQVNEKI NQSLAFIRKS DELLGSGGSG 480 SGEKAAKAEE AARKMEELFK KHKIVAVLRA NSVVEAAIEKA VAVFAGGVHL IEITFTVPDA 540 DTVIKALSVL KEKGAIIGAG TVTSEQCRK AVESGAEFIV SPHLDEEISQ FCKEKGVFYM 600 PGVMPTELV KAMKLGHTIL KLFPGEVVGP QFVKAMKGPF PNVKFVPTGG VNLDNVCEWF 660 KAGVLAVGVG SALVKGTPDE VREKAKAFVE KIRGCTE 697	
SEQ ID NO: 89 moltype = AA length = 736 FEATURE Location/Qualifiers REGION 1..736 note = sc9-10 DS-Cav1 A149C Y458C S46G K465Q S215P E92D -foldon-I53-50A embodiment VARIANT 1..25 note = optionally absent VARIANT 469..474 note = optionally absent source 1..736 mol_type = protein organism = synthetic construct	
SEQUENCE: 89 MELLILKANA ITTILTAVTF CFASQGNITE EFYQSTCSAV SKGYLGALRT GWYTSVITIE 60 LSNIKENKCN GTDAVKLIK QELDKYKNAV TDLQLLMQST PATGSGSAIC SGVAVCKVLH 120 LEGEVNKIKS ALLSTNKAVV SLSNGVSVLT FKVLDLKNYI DKQLLPILNK QSCSIPNIET 180 VIEFQQKNRR LLEITREFSV NAGVTPVST YMMLTNSELLS LINDDMPITND QKKLMSNNVQ 240 IVRQQSYSIM CIIKEEVLAY VVQLPLYGVI DTPCWKLHTS PLCTTNTKEG SNICLRTD 300 GWYCDNAGSV SFFPQAETCK VQSNRVCFTD MNSRTLPSVE NLCNVDIIFNP KYDCKIMTSK 360 TDVSSSVITS LGAIIVSCYKG TKCTASNKR GIICKTFSNCG DYVSNKGVDT VSVGNTLYCV 420 NKQEGQSLYV KGEPIINFYD PLVFPDSDEFD ASISQVNEKI NQSLAFIRKS DELLGYIPEA 480 PRDGQAYVRK DGEWVLLSTF LGGSHHHHHH HHHGGSGGSG SEKAAKAEEA ARKMEELFK 540 KHKIVAVLRA NSVVEAAIEKA VAVFAGGVHL IEITFTVPAD TVIKALSVL EKGAIIGAGT 600 VTSVEQCRKA VESGABEFIV PHLDEEISQ CKEKGVFYM GMVMTPELVK AMKLGHITLK 660 LFPGEVGVGPQ FVKAMKGPF PNVKFVPTGG VNLDNVCEWFK AGVLAVGVGS ALVKGTPDEV 720 REKAKAFVEK IRGCTE 736	
SEQ ID NO: 90 moltype = AA length = 697 FEATURE Location/Qualifiers REGION 1..697 note = sc9-10 DS-Cav1 A149C Y458C S46G K465Q S215P E92D - F10 embodiment VARIANT 1..25 note = optionally absent VARIANT 469..474 note = optionally absent source 1..697 mol_type = protein organism = synthetic construct	
SEQUENCE: 90 MELLILKANA ITTILTAVTF CFASQGNITE EFYQSTCSAV SKGYLGALRT GWYTSVITIE 60 LSNIKENKCN GTDAVKLIK QELDKYKNAV TDLQLLMQST PATGSGSAIC SGVAVCKVLH 120 LEGEVNKIKS ALLSTNKAVV SLSNGVSVLT FKVLDLKNYI DKQLLPILNK QSCSIPNIET 180 VIEFQQKNRR LLEITREFSV NAGVTPVST YMMLTNSELLS LINDDMPITND QKKLMSNNVQ 240 IVRQQSYSIM CIIKEEVLAY VVQLPLYGVI DTPCWKLHTS PLCTTNTKEG SNICLRTD 300 GWYCDNAGSV SFFPQAETCK VQSNRVCFTD MNSRTLPSVE NLCNVDIIFNP KYDCKIMTSK 360 TDVSSSVITS LGAIIVSCYKG TKCTASNKR GIICKTFSNCG DYVSNKGVDT VSVGNTLYCV 420 NKQEGQSLYV KGEPIINFYD PLVFPDSDEFD ASISQVNEKI NQSLAFIRKS DELLGSGGSG 480 SGEKAAKAEE AARKMEELFK KHKIVAVLRA NSVVEAAIEKA VAVFAGGVHL IEITFTVPDA 540	

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DTVIKALSVL KEKGAIIGAG TVTSVEQCRK AVESGAEFIV SPHLDEEISQ FCKEKGVFYM	600
PGVMTPTELV KAMKLGHIL KLFPGEVVGP QFVKAMKGF PNVKFVPTGG VNLDNVCEWF	660
KAGVLAVGVG SALVKGTPDE VREKAKAFVE KIRGCTE	697

SEQ ID NO: 91 moltype = AA length = 753

FEATURE Location/Qualifiers
 REGION 1..753
 note = SC-DM (N67I, S215P) - foldon-I53-50A embodiment
 VARIANT 1..25
 note = optionally absent
 VARIANT 486..491
 note = optionally absent
 source 1..753
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 91

MELLILKANA ITTILTAVTF CFASQGNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE	60
LSNIKKIKCN GTDAKIKLIK QELDKYKNAV TELQLLMQST PATNNQARGS GSGRSLGFLL	120
GVGSIAISGV AVSKVLHLEG EVNKKIKSALL STNKAVVSLs NGVSVLTSKV LDLKNYIDKQ	180
LLPIVNQKSC SIPNIETVIE FQQKNNRRLIE ITREFSVNAG VTTPVSTYML TNSELLSLIN	240
DMPITNDQKK LMSNNVQIVR QQSYSIMSII KEEVLAYVQQ LPLYGVIDTP CWKLHTSPLC	300
TTNTKEGSNI CLTRTDRGWY CDNAGSVSFF PQAETCKVQS NRVFCDTMNS LTLPSEVNLC	360
NVDIFNPKYD CKIMTSKTDV SSSVITSGLA IVSCYGKTKC TASNKNRGII KTFNSNGCDYV	420
SNKGVDTSV GNTLYYVVKQ EGKSLYVKG E PIINFYDPLV FPSDEFDASI SQVNEKINQS	480
LAFIRKSDEL LGYIPEAPRD GQAYVRKDGE WVLLSTFLGS GSHHHHHHHH GGSGGSGSEK	540
AAKAEEAARK MEELFKKKH VAVLTRANSVE EAIEKAVAVF AGGVHLLIEIT FTVPADTVI	600
KALSVLKEKG AIIAGTGTVS VEQCRKAVES GAEFIVSPHL DEEISQFCKE KGVFYMPGVM	660
TPTELVKAMK LIGHТИLKF GEVVGPFQVK AMKGPFPPNVK FVPTGGVNLD NVCEWFKAGV	720
LAVVGVSALV KGTPDEVREK AKAFVEKIRG CTE	753

SEQ ID NO: 92 moltype = AA length = 714

FEATURE Location/Qualifiers
 REGION 1..714
 note = SC-DM (N67I, S215P) - F10 embodiment
 VARIANT 1..25
 note = optionally absent
 VARIANT 486..491
 note = optionally absent
 source 1..714
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 92

MELLILKANA ITTILTAVTF CFASQGNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE	60
LSNIKKIKCN GTDAKIKLIK QELDKYKNAV TELQLLMQST PATNNQARGS GSGRSLGFLL	120
GVGSIAISGV AVSKVLHLEG EVNKKIKSALL STNKAVVSLs NGVSVLTSKV LDLKNYIDKQ	180
LLPIVNQKSC SIPNIETVIE FQQKNNRRLIE ITREFSVNAG VTTPVSTYML TNSELLSLIN	240
DMPITNDQKK LMSNNVQIVR QQSYSIMSII KEEVLAYVQQ LPLYGVIDTP CWKLHTSPLC	300
TTNTKEGSNI CLTRTDRGWY CDNAGSVSFF PQAETCKVQS NRVFCDTMNS LTLPSEVNLC	360
NVDIFNPKYD CKIMTSKTDV SSSVITSGLA IVSCYGKTKC TASNKNRGII KTFNSNGCDYV	420
SNKGVDTSV GNTLYYVVKQ EGKSLYVKG E PIINFYDPLV FPSDEFDASI SQVNEKINQS	480
LAFIRKSDEL LGSGGSGSEK KAAKAEEAAR KMELFKKKH IVAVLTRANSV EEAIEKAVAV	540
FAGGVHLLIEI TFTVPADTV I KALSVLKEKG GAIIGAGTGTVS SVEQCRKAVE SGAEFIVSPH	600
LDEEISQFC KEGVFYMPGV MTPTELVKAM KLGHТИLKF GEVVGPFQVK KAMKGPFPPNV	660
KFVPTGGVN DNVCEWFKAG VLAVVGVSALV VKGTPDEVRE KAKAFVEKIR GCTE	714

SEQ ID NO: 93 moltype = AA length = 753

FEATURE Location/Qualifiers
 REGION 1..753
 note = SC-TM (N67I, S215P, and E487Q) - foldon-I53-50A embodiment
 VARIANT 1..25
 note = optionally absent
 VARIANT 486..491
 note = optionally absent
 source 1..753
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 93

MELLILKANA ITTILTAVTF CFASQGNITE EFYQSTCSAV SKGYLSALRT GWYTSVITIE	60
LSNIKKIKCN GTDAKIKLIK QELDKYKNAV TELQLLMQST PATNNQARGS GSGRSLGFLL	120
GVGSIAISGV AVSKVLHLEG EVNKKIKSALL STNKAVVSLs NGVSVLTSKV LDLKNYIDKQ	180
LLPIVNQKSC SIPNIETVIE FQQKNNRRLIE ITREFSVNAG VTTPVSTYML TNSELLSLIN	240
DMPITNDQKK LMSNNVQIVR QQSYSIMSII KEEVLAYVQQ LPLYGVIDTP CWKLHTSPLC	300
TTNTKEGSNI CLTRTDRGWY CDNAGSVSFF PQAETCKVQS NRVFCDTMNS LTLPSEVNLC	360
NVDIFNPKYD CKIMTSKTDV SSSVITSGLA IVSCYGKTKC TASNKNRGII KTFNSNGCDYV	420
SNKGVDTSV GNTLYYVVKQ EGKSLYVKG E PIINFYDPLV FPSDEFDASI SQVNEKINQS	480

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LAFIRKSDEL	LGYIPEAPRD	GQAYVRKDGE	WVLLSTFLGS	GSHHHHHHHH	GGSGGGSEK	540
AKAAEEAARK	MEELFKKKHKL	VAVLTRANSVE	EAEIKAVAVF	AGGVHLIEIT	FTVPDADTVI	600
KALSVLKKG	AIIGAGTVTS	VEQCRKAVES	GAEFIVSPHL	DEEISQFCKE	KGVFYMPGVM	660
TPTELVKAMK	LGHTILKLFP	GEVVGQPQVK	AMKGPFPNVK	FVPTGGVNLD	NVCEWFKA	720
LAVGVGSALV	KGTPDEVREK	AKAFVEKIRG	CTE			753

SEQ ID NO: 94	moltype = AA	length = 714
FEATURE	Location/Qualifiers	
REGION	1..714	note = SC-TM (N67I, S215P, and E487Q) - I53-50A - F10 embodiment
VARIANT	1..25	note = optionally absent
VARIANT	486..491	note = optionally absent
source	1..714	mol_type = protein organism = synthetic construct

SEQUENCE: 94						
MELLILKANA	ITTIILTAVTF	CFASGQNITE	EFYQSTCSAV	SKGYLSALRT	GWYTSVITIE	60
LSNIKKIKCN	GTDAKIKLIK	QELDKYKNAV	TELQLLMQST	PATNNQARGS	GSGRSLGFLL	120
GVSSAIASGV	AVSKVLHLEG	EVNKIKSALL	STNKAVVSSL	NGVSVLTSKV	LDLKNYIDKQ	180
LLPIVNDQSC	SPNVIETQIV	FQQKNNRRLLE	ITREFPSVNAQ	VTPVSTYML	TNSELLSLIN	240
DMPITNDQSC	LMSNNVQIVR	QOSYSIMSII	KEEVLAYVQQ	LPLYGVIDTP	CWKLHTSPCLC	300
TTNTKEGSNI	CLTRTDRGWY	CDNAGSVSFF	PQAETCKVQS	NRVFCDTMNS	LTLPESEVNL	360
NVDIFNPKYD	CKIMTSKTDV	SSSVITSLGA	I1VSCYGKTKC	TASNKNRGII	KTFNSNGCDYV	420
SNKGVDTVSV	GNTLYVNQK	EGKSGVYVKE	PIINFYDPLV	FPSDQFDASI	SQVNEKINQS	480
LAFIRKSDEL	LGSGGGSGSE	AKAAEEAAR	KMEELFKKKH	IVAVLTRANSV	EEAIEKAVAV	540
FAGGVHLIEI	TFTVVDADTV	IKALSVLKEK	AIIGAGTVT	SVEQCRKAVE	SGAEFIVSPH	600
LDEEISQFC	EKGVFYMPGV	MTPTELVKAM	KLGHТИKLFP	PGEVVGQPQVK	KAMKGPFPNV	660
KFVPTGGVN	DNVCEWFKA	VLAvgvgsal	VKGTPDEVRE	AKAFVEKIR	GCTE	714

SEQ ID NO: 95	moltype = AA	length = 752
FEATURE	Location/Qualifiers	
REGION	1..752	note = HMPV-F with A113C, A339C, T160F, I177L - foldon-I53-50A embodiment
VARIANT	1..18	note = optionally absent
source	1..752	mol_type = protein organism = synthetic construct

SEQUENCE: 95						
MSWKVVIIIFS	LLITPQHGLK	ESYLEECSCT	ITEGYLSVLR	TGWTNTVFTL	EVGDVENLTC	60
SDGPSLIKTE	LDLTKSALRE	LKTVDADQLA	REEQIENPRQ	SRFVLGAIAL	GVCTAAAVTA	120
GVIAIAKTI	ESEVTAIKNA	LKTTENEAVST	LGNGVRVLA	AVRELKDFVS	KNLTRALNKN	180
KCDIDDLKMA	VSFSQFNRRF	LNVNVRQFSDN	AGITPAISL	LMTDAELARA	VSNMPTSAGQ	240
IKLMLENRAM	VRRKGPFGILI	GVYGSVSIYM	VQLPIFGVID	TPCWIVVKAAP	SCSGKGNYA	300
CLLRDQGWY	CONAGSTVYY	PNEKDCETRG	DHVFCDTACG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPPLGA	LVACYKGVSC	SIGSNRVGII	KQLNKGCYI	TNQDADTVTI	420
DNTVYQLSKV	EQEIQHVIKGR	PVSSSFDPPIK	FPEDQFNVAL	DQVFENIENS	QALVDQSNRI	480
LSSAEKGNTG	GYIPEAPRPG	QAYVTKGEW	VLLSTFLGSR	SHHHHHHHHG	GGSGSGSEKA	540
AKAAEEAARKM	EELFKKKHIV	AVLTRANSWE	AIEKAVAVFA	GGVHLIEITF	TVPDADTVIK	600
ALSLVKEKG	IIGAGTVTSV	EQCRKAVESG	AEFIVSPHL	EEISQFCKEK	KGVFYMPGVM	660
PTELVKAMKL	GHTILKLFP	EVVGPQFVKA	MKGPFPNVK	FVPTGGVNLDN	VCEWFKA	720
AVGVGSALVK	GTPDEVREKA	KAFVEKIRG	TE			752

SEQ ID NO: 96	moltype = AA	length = 713
FEATURE	Location/Qualifiers	
REGION	1..713	note = HMPV-F with A113C, A339C, T160F, I177L-I53-50A F10 embodiment
VARIANT	1..18	note = optionally absent
source	1..713	mol_type = protein organism = synthetic construct

SEQUENCE: 96						
MSWKVVIIIFS	LLITPQHGLK	ESYLEECSCT	ITEGYLSVLR	TGWTNTVFTL	EVGDVENLTC	60
SDGPSLIKTE	LDLTKSALRE	LKTVDADQLA	REEQIENPRQ	SRFVLGAIAL	GVCTAAAVTA	120
GVIAIAKTI	ESEVTAIKNA	LKTTENEAVST	LGNGVRVLA	AVRELKDFVS	KNLTRALNKN	180
KCDIDDLKMA	VSFSQFNRRF	LNVNVRQFSDN	AGITPAISL	LMTDAELARA	VSNMPTSAGQ	240
IKLMLENRAM	VRRKGPFGILI	GVYGSVSIYM	VQLPIFGVID	TPCWIVVKAAP	SCSGKGNYA	300
CLLRDQGWY	CONAGSTVYY	PNEKDCETRG	DHVFCDTACG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPPLGA	LVACYKGVSC	SIGSNRVGII	KQLNKGCYI	TNQDADTVTI	420
DNTVYQLSKV	EQEIQHVIKGR	PVSSSFDPPIK	FPEDQFNVAL	DQVFENIENS	QALVDQSNRI	480

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LSSAEKGNTG	GSGGSGSGEK	AAKAAAARK	MEELFKHHKI	VAVLTRANSVE	EAIEKAVAVF	540
AGGVHLIEIT	PTVVDADTVI	KALSVLKEKG	AIIGAGTVTS	VEQCRKAVES	GAEFIVSPHL	600
DEEISQFCKE	KGVFYMPGVM	PTPELVKAMK	LGHTILKLFP	GEVVGPQFVK	AMKGPFPNVK	660
FVPTGGVNLD	NVCEWFKAGV	LAVGVGSALV	KGTPDEVREK	AKAFVEKIRG	CTE	713

SEQ ID NO: 97 moltype = AA length = 752

FEATURE Location/Qualifiers
REGION 1..752
 note = HMPV-F with A113C, A120C, A339C, T160F, I177L, and
 Q426C - foldon-I53-50A embodiment

VARIANT 1..18
 note = optionally absent
source 1..752
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 97

MSWKVVIIFS	LLITPQHGLK	ESYLEESCST	ITEGRYLSVLR	TGWYTNVFTL	EVGDVENLTC	60
SDGPSLIKTE	LLDLTKSALRE	LKTVSADQLA	REBQIENPRQ	SRFVLGAIAL	GVCTAAAVTC	120
GVIAIKTIRL	ESEVTAIKNA	LKTTNEAVST	LGNGVRVLAF	AVRELKDFVS	KNLTRALNKN	180
KCDIDDLKMA	VSFSQFNRRF	LNVVRQFSND	AGITPAISLD	LMTDAELARA	VSNMPTSAGQ	240
IKLMLENRAM	VRRKGFGLI	GVYGSVVIYM	VQLPIFGVID	TPCWIVKAAP	SCSGKKGNYA	300
CLLRDQGWY	CQNAGSTVYY	PNEKDCETRG	DHVFCDTACG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPLGA	LVACYKGVC	SIGSNRVGII	KQLNKGCSSI	TNQDADTVTI	420
DNTVYCLSKV	EQEIQHVIKGR	PVSSSFDPK	FPEDQFNVAL	DQVFENIENS	QALVDQSNRI	480
LSSAEKGNTG	YIPEAPRDG	QAYVRKDGEW	VLLSTFLGSG	SHHHHHHHHG	GSGGSGSEKA	540
AAKAAAARKM	EELFKHHKIV	AVLTRANSVE	AIEKAVAVFA	GGVHLIEITF	TPVVDADTVI	600
ALSLVLEKGA	IIGAGTVTSV	EQCRKAVESG	AEIFIVSPHL	EEISQFCKEK	GVFYMPGVMT	660
PTELVKAMKL	GHTILKLFPG	EVGVGPQFVK	MKGPFPPNVK	VPTGGVNLDN	CEWFKAGVL	720
AVGVGSALVK	GTPDEVREKA	KAFVEKIRG	TE			752

SEQ ID NO: 98 moltype = AA length = 713

FEATURE Location/Qualifiers
REGION 1..713
 note = HMPV-F with A113C, A120C, A339C, T160F, I177L, and
 Q426C - F10 embodiment

VARIANT 1..18
 note = optionally absent
source 1..713
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 98

MSWKVVIIFS	LLITPQHGLK	ESYLEESCST	ITEGRYLSVLR	TGWYTNVFTL	EVGDVENLTC	60
SDGPSLIKTE	LLDLTKSALRE	LKTVSADQLA	REBQIENPRQ	SRFVLGAIAL	GVCTAAAVTC	120
GVIAIKTIRL	ESEVTAIKNA	LKTTNEAVST	LGNGVRVLAF	AVRELKDFVS	KNLTRALNKN	180
KCDIDDLKMA	VSFSQFNRRF	LNVVRQFSND	AGITPAISLD	LMTDAELARA	VSNMPTSAGQ	240
IKLMLENRAM	VRRKGFGLI	GVYGSVVIYM	VQLPIFGVID	TPCWIVKAAP	SCSGKKGNYA	300
CLLRDQGWY	CQNAGSTVYY	PNEKDCETRG	DHVFCDTACG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPLGA	LVACYKGVC	SIGSNRVGII	KQLNKGCSSI	TNQDADTVTI	420
DNTVYCLSKV	EQEIQHVIKGR	PVSSSFDPK	FPEDQFNVAL	DQVFENIENS	QALVDQSNRI	480
LSSAEKGNTG	GSGGSGSGEK	AAKAAAARK	MEELFKHHKI	AVLTRANSVE	EAIEKAVAVF	540
AGGVHLIEIT	PTVVDADTVI	KALSVLKEKG	AIIGAGTVTS	VEQCRKAVES	GAEFIVSPHL	600
DEEISQFCKE	KGVFYMPGVM	PTPELVKAMK	LGHTILKLFP	GEVVGPQFVK	AMKGPFPPNVK	660
FVPTGGVNLD	NVCEWFKAGV	LAVGVGSALV	KGTPDEVREK	AKAFVEKIRG	CTE	713

SEQ ID NO: 99 moltype = AA length = 751

FEATURE Location/Qualifiers
REGION 1..751
 note = HMPV-F 115-BV (A185P) -foldon-I53-50A embodiment

VARIANT 1..18
 note = optionally absent
source 1..751
 mol_type = protein
 organism = synthetic construct

SEQUENCE: 99

MSWKVVIIFS	LLITPQHGLK	ESYLEESCST	ITEGRYLSVLR	TGWYTNVFTL	EVGDVENLTC	60
ADGPSLIKTE	LLDLTKSALRE	LRTVSADQLA	REBQIENPR	RRFVLGAIAL	GVATAAAVTA	120
GVIAIKTIRL	ESEVTAIKNA	LKKTNEAVST	LGNGVRVLAT	AVRELKDFVS	KNLTRAINKN	180
KCDIPDLKMA	VSFSQFNRRF	LNVVRQFSND	AGITPAISLD	LMTDAELARA	VSNMPTSAGQ	240
IKLMLENRAM	VRRKGFGLI	GVYGSVVIYM	VQLPIFGVID	TPCWIVKAAP	SCSEKGNYA	300
CLLRDQGWY	CQNAGSTVYY	PNEKDCETRG	DHVFCDTAAG	INVAEQSKEC	NINISTTNYP	360
CKVSTGRHPI	SMVALSPLGA	LVACYKGVC	SIGSNRVGII	KQLNKGCSSI	TNQDADTVTI	420
DNTVYQLSKV	EQEIQHVIKGR	PVSSSFDPVK	FPEDQFNVAL	DQVFESIENS	QALVDQSNRI	480
LSSAEKGNTG	YIPEAPRDGQ	AYVVRKDGEW	LLSTFLGS	HHHHHHHHHG	GGGSGSEKAA	540
AAKAAAARKM	EELFKHHKIV	VLRANSVEA	IIEKAVAVFAG	GGVHLIEITF	TPVVDADTVI	600
LSVLKEKGAI	IIGAGTVTSV	QCRAKAVESGA	EFIGVSPHLDE	EISQFCKEKG	GVFYMPGVMT	660
TELVKAMKLG	HTILKLFPGE	EVGVGPQFVK	MKGPFPPNVK	VPTGGVNLDN	CEWFKAGVLA	720

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VGGSALVKG TPDEVREKAK AFVEKIRGCT E	751
SEQ ID NO: 100	moltype = AA length = 712
FEATURE	Location/Qualifiers
REGION	1..712
VARIANT	note = HMPV-F 115-BV (A185P)-I53-50A - F10 embodiment 1..18
source	note = optionally absent 1..712 mol_type = protein organism = synthetic construct
SEQUENCE: 100	
MSWKVVIIFS LLITPQHGLK ESYLEESCST ITEGYLSVLR TGWYTNVFTL EVGDVENLTC 60	
ADGPSLITE LDDTKSALRE LRTVSADQLA REEQIENPRR RRFVLGAIAL GVATAAAVTA 120	
GVAIAKTI RL ESEVTAIKNA LKKTNEAVST LGNGVRVLAT AVRELKDFVS KNLTRAIKN 180	
KCDIPDLKMA VSFSQFNRRF LNVVRQFSDN AGITPAISLD LMTDAELARA VSNMPTSAGQ 240	
I KLMLENRAM VRRKGFGILI GVGSSVIIYM VOLPIFGVID TPCWIVKAAP SCSEKKGNYA 300	
CLLRDQGWY CQNAGSTVYY PNEKDCETRG DHVFCDTAAG INVAEQSKEC NINISTTNYP 360	
CKVSTGRHPI SMVALSPLGA LVACYKGVC SIGSNRVGII KQLNKGCYI TNQDADTVTI 420	
DNTVYQLSKV EGEQHVIKGR PVSSSFDPVK FPEDQFNVAL DQVFESIENS QALVDQSNRI 480	
LSSAEKGNTG SGGSGSGEKA AKAEEAARKM EELFKHHKIV AVLTRANSVEE AIEKAVAVFA 540	
GGVHLIEITF TVPDADTVIK ALSVLKEKGIA IIGAGTGTTSV EQCRKAVESG AEFIVSPHLD 600	
EEISQFCKEK GFVYMPGVMT PTELVKAMKL GHTILKLFPG EVVGPQFVKA MKGPFPNVKF 660	
VPTGGVNLDN VCEWFKAGVL AVGGSALVK GTPDEVREKA KAFVEKIRGC TE 712	
SEQ ID NO: 101	moltype = AA length = 490
FEATURE	Location/Qualifiers
REGION	1..490
VARIANT	note = HMPV F AAK62968.2 fusion protein [Human metapneumovirus] 1..18
source	note = optionally absent 1..490 mol_type = protein organism = synthetic construct
SEQUENCE: 101	
MSWKVVIIFS LLITPQHGLK ESYLEESCST ITEGYLSVLR TGWYTNVFTL EVGDVENLTC 60	
ADGPSLITE LDDTKSALRE LRTVSADQLA REEQIENPRR SRFVLGAIAL GVATAAAVTA 120	
GVAIAKTI RL ESEVTAIKNA LKKTNEAVST LGNGVRVLAT AVRELKDFVS KNLTRAIKN 180	
KCDIADLKMA VSFSQFNRRF LNVVRQFSDN AGITPAISLD LMTDAELARA VSNMPTSAGQ 240	
I KLMLENRAM VRRKGFGFLT GVGSSVIIYM VOLPIFGVID TPCWIVKAAP SCGKGNYA 300	
CLLRDQGWY CQNAGSTVYY PNEKDCETRG DHVFCDTAAG INVAEQSKEC NINISTTNYP 360	
CKVSTGRHPI SMVALSPLGA LVACYKGVC SIGSNRVGII KQLNKGCYI TNQDADTVTI 420	
DNTVYQLSKV EGEQHVIKGR PVSSSFDPVK FPEDQFNVAL DQVFESIENS QALVDQSNRI 480	
LSSAEKGNTG 490	

1-43. (canceled)

44. 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; 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; and

wherein the one or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof, comprise:

a polypeptide having at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identity to a polypeptide selected from the group consisting of

- (i) SEQ ID NO: 53 in which residues 1-25 are absent;
- (ii) SEQ ID NO: 61 in which residues 1-25 are absent;

(iii) SEQ ID NO: 62 in which residues 1-25 are absent;

(iv) SEQ ID NO: 63 in which residues 1-25 are absent;

(v) SEQ ID NO: 64 in which residues 1-25 are absent;

(vi) SEQ ID NO: 65 in which residues 1-18 are absent;

(vii) SEQ ID NO: 66 in which residues 1-18 are absent;

(viii) SEQ ID NO: 67 in which residues 1-18 are absent;

(ix) SEQ ID NO: 68 in which residues 1-18 are absent;
or

(x) SEQ ID NO: 101 in which residues 1-18 are absent.

45. The nanostructure of claim **44**, wherein the polypeptide comprises one of SEQ ID NO:53 and 61-64 in which residues 1-25 are absent and the polypeptide comprise one or more of the following residues: 67I, 149C, 458C, 246G, 465Q, 215P, 92D, and 487Q.

46. The nanostructure of claim **45**, wherein the polypeptide comprises one of the following amino acid substitutions: A149C and Y458C; A149C, Y458C, S46G, K465Q, S215P, and E92D; N67I and S215P; or N67I, S215P, and E487Q.

47. The nanostructure of claim **45**, wherein the polypeptide comprises one of SEQ ID NO:65-68 and 101 in which

residues 1-18 are absent and the polypeptide comprise one or more of the following residues: 113C, 120C, 339C, 160F, 177L, 185P, and 426C.

48. The nanostructure of claim **47**, wherein the polypeptide comprises one of the following amino acid substitutions: A185P; A113C, A339C, T160F, and 1177L; or A113C, A120C, A339C, T160F, 1177L, and Q426C.

49. The nanostructure of claim **44**, 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 and/or as a fusion protein with the second polypeptides.

50. The nanostructure of claim **49**, wherein the plurality of first assemblies each comprise identical fusion proteins; the plurality of first assemblies in total comprise two or more paramyxovirus and/or pneumovirus F proteins, or antigenic fragments thereof; or only a subset of the first polypeptides comprises a fusion protein with an F protein or antigenic fragment thereof.

51. The nanostructure of claim **49**, 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.

52. The nanostructure of claim **51**, wherein the amino acid linker sequence comprises one or more trimerization domain, the amino acid sequence GYIPEAPRDGQAY-VRKDGEWLLSTFL (SEQ ID NO:54), or comprises a Gly-Ser linker.

53. The nanostructure of claim **44**, wherein each first assembly comprises a homotrimer of the first polypeptide.

54. The nanostructure of claim **44**, wherein:

(i) (a) the first 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 a polypeptide selected from the group consisting of SEQ ID NOS:1-51; and

(b) 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 a polypeptide selected from the group consisting of SEQ ID NOS:1-51; or

(ii) wherein the first 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 T33-31A (SEQ ID NO:51) 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 T33-09B/T33-31B (SEQ ID NO:44); or

wherein the first 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 T33-15B (SEQ ID NO:46) 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 T33-15A (SEQ ID NO:45); or

wherein the first 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 selected from the group consisting of I53-50A (SEQ ID NO:7), I53-50A.1 (SEQ ID NO:29), I53-50A.1NegT2 (SEQ ID NO:30), and I53-50A.1PosT1 (SEQ ID NO:31), 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 selected from the group consisting of I53-50B (SEQ ID NO:8), I53-50B.1 (SEQ ID NO:32), I53-50B.1NegT2 (SEQ ID NO:33), and I53-50B.4PosT1 (SEQ ID NO:34); or

wherein the first 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 I32-28A (SEQ ID NO:21) 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 I32-28B (SEQ ID NO:22).

55. An immunogenic composition comprising the nanostructure of claim **44**, and a pharmaceutically acceptable carrier, optionally, further comprising an adjuvant.

56. A method for generating an immune response to paramyxovirus and/or pneumovirus F protein in a subject, and/or for treating or limiting a paramyxovirus and/or pneumovirus infection in a subject, the method comprising administering to the subject in need thereof an effective amount of the nanostructure of claim **44** to generate the immune response and/or to treat or limit a paramyxovirus and/or pneumovirus infection in the subject.

57. The method of claim **56**, wherein the administering results in production of paramyxovirus and/or pneumovirus neutralizing antibodies in the subject, optionally, wherein the neutralizing antibodies are present in sera of the subject at a titer (1/ID50) of at least 9,400.

58. A process for assembling the nanostructures of claim **44** in vitro, comprising mixing two or more nanostructure components in aqueous conditions to drive spontaneous assembly of the desired nanostructure.

59. The process of claim **58**, wherein:

i) the mixing comprises mixing first assemblies comprising first polypeptides each comprising an F protein or antigenic fragment thereof with second assemblies comprising second polypeptides in an approximately 1:1 molar first polypeptide: second polypeptide ratio under conditions and for a time suitable to permit interaction of the first assemblies and the second assemblies to form the nanostructure;

ii) the mixing comprises mixing first assemblies comprising first polypeptides, wherein fewer than all first polypeptides comprise an F protein with second assemblies comprising second polypeptides in an approximately 1:1 first polypeptide: second polypeptide molar ratio under conditions and for a time suitable to permit interaction of the first assemblies and the second assemblies to form the nanostructure; or

iii) the mixing comprises mixing first assemblies comprising first polypeptides each comprising an F protein, wherein in total the first polypeptides comprise mul-

tiple different F proteins with second assemblies comprising second polypeptides in an approximately 1:1 molar first polypeptide: second polypeptide ratio under conditions and for a time suitable to permit interaction of the first assemblies and the second assemblies to form the nanostructure comprising multiple F proteins, or antigenic fragments thereof.

60. The process of claim **58**, wherein the mixing comprises mixing first assemblies comprising first polypeptides each comprising an F protein, wherein in total the first polypeptides comprise multiple different F proteins with second assemblies comprising second polypeptides in an approximately 1:1 molar first polypeptide: second polypeptide ratio under conditions and for a time suitable to permit interaction of the first assemblies and the second assemblies to form the nanostructure comprising multiple F proteins, or antigenic fragments thereof.

61. A recombinant nucleic acid encoding the fusion protein as recited in claim **49**, optionally the recombinant nucleic acid is operatively linked to a promoter.

62. A recombinant host cell, comprising the recombinant nucleic acid of claim **61**.

63. A recombinant host cell, comprising one or more recombinant expression vectors capable of expressing the first polypeptides and the second polypeptides of claim **44**.

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