

PROTEIN-BASED NANOPARTICLE VACCINE FOR METAPNEUMOVIRUS**CROSS-REFERENCE TO RELATED APPLICATION**

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 63/113,686, filed November 13, 2020, which is incorporated by reference herein in its entirety.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted in ASCII format via EFS-WEB and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 5, 2021 is named ICVX-011_01WO_SeqList.txt and is 442,995 bytes in size.

BACKGROUND

[0003] Human metapneumovirus (hMPV) causes upper and lower respiratory disease in people of all ages. The symptoms can include cough, fever, nasal congestion, and shortness of breath. In some cases, hMPV infection can progress to severe disease, such as bronchitis or pneumonia.

[0004] hMPV is single-stranded RNA virus in the paramyxovirus family, which also includes measles, mumps, and other respiratory tract infections such as respiratory syncytial virus (RSV). hMPV can be of two subtypes, A and B that are identified by genotyping the G and F genes in the viral genome. The F gene encodes hMPV fusion glycoprotein (hMPV F protein) and may be used as a target for vaccine development. However, to date there has been no approved vaccine for hMPV in humans despite the clinical need.

[0005] There is therefore a need for a vaccine against hMPV.

SUMMARY

[0006] Provided are protein-based virus-like particle (VLP) vaccines for human metapneumovirus in which the hMPV F protein ectodomain is linked to, and thereby displayed on, a protein-based VLP.

[0007] In one aspect, the disclosure provides a virus-like particle (VLP), comprising a first component and optionally a second component, wherein the first component is a fusion protein, comprising a human metapneumovirus (hMPV) F protein ectodomain or antigenic variant

thereof, and a first multimerization domain; and wherein the second component, if present, is a protein comprising a second multimerization domain.

[0008] In some embodiments, first multimerization domain is a trimerization domain. In some embodiments, the multimerization domain is selected from SEQ ID NOS: 1, 4, 5, 7, 9, 18, 19, 21, 24, 25, 26, 29, 30, 31, 34, 36, 37, 39, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 144, or 145, or functional variants thereof. In some embodiments, the multimerization domain is I53-50A (SEQ ID NO: 7 or SEQ ID NO: 144) or a functional variant or variant thereof. In some embodiments, the first multimerization domain shares at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity to SEQ ID NO: 7, SEQ ID NO: 53, SEQ ID NO: 144, or SEQ ID NO: 145. In some embodiments, the first multimerization domain comprises: the amino acid substitutions C74A and C98A; the amino acid substitutions C163A and C201A; or the amino acid substitutions C74A, C98A, C163A, and C201A relative to SEQ ID NO: 144. In some embodiments, the first multimerization domain has a polypeptide sequence identical to SEQ ID NO: 7 or SEQ ID NO: 144. In some embodiments, the first multimerization domain has a polypeptide sequence identical to SEQ ID NO: 53 or SEQ ID NO: 145. In some embodiments, the first multimerization domain is a VLP-forming domain. In some embodiments, the first multimerization domain is adapted to drive assembly of an icosahedral particle or tetrahedral particle, optionally with a second multimerization domain.

[0009] In some embodiments, second multimerization domain is selected from SEQ ID NOs: 2, 3, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 20, 22, 23, 27, 28, 32, 33, 35, 38, 40, and 41 or functional variants and fragments thereof. In some embodiments, the second multimerization domain is I53-50B (SEQ ID NO: 8), I53-50B.4PosT1 (SEQ ID NO: 34), or a functional variant thereof.

[0010] In some embodiments, the first component and the second component are joined by a linker sequence. In some embodiments, the linker sequence comprises a foldon, wherein the foldon sequence is EKAAKAEEAARK (SEQ ID NO: 125). In some embodiments, the linker sequence comprises GSGGSGSGSGGS (SEQ ID NO: 126).

[0011] In some embodiments, the hMPV F protein ectodomain contains one or more substitutions or deletions selected from A185P, Q100R, S101R, T127C, N153C, V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, G106 deletion, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, I177L, K450A, S470A, G294E, T365C, V463C, L219K, H368N, and/or V231I. In some embodiments, the hMPV F protein ectodomain contains one or more substitutions or deletions

selected from V84C, A140C, A147C, N97G, P98G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, R99G, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, Q100R, S101R, G106 deletion, S101R, A185P, I177L, and/or G294E.

[0012] In some embodiments, the hMPV F protein ectodomain contains two or more substitutions or deletions selected from V84C, A140C, A147C, N97G, P98G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, R99G, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, Q100R, S101R, G106 deletion, S101R, A185P, I177L, and/or G294E. In some embodiments, the hMPV F protein ectodomain contains one or more substitutions selected from A185P, Q100R, S101R, T127C, N153C, T365C, V463C, L219K, V231I, G294E, N153C, N97G, P98G, R99G, Q100G, S101G, H368N, and/or R102G.

[0013] In some embodiments, the hMPV F protein ectodomain contains two or more of A185P, Q100R, S101R, T127C, N153C, T365C, V463C, L219K, V231I, G294E, N153C, N97G, P98G, R99G, Q100G, S101G, H368N, and/or R102G. In some embodiments, the hMPV F protein ectodomain comprises: the substitutions Q100R and S101R; the substitutions A185P, Q100R, and S101R; the substitutions A185P, T127C, N153C, Q100R, and S101R; the substitutions V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, and R102G; the substitutions A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, and R102G; the substitutions and deletion A63C, A140C, A147C, K188C, G106 deletion, N97G, P98G, R99G, Q100G, S101G, and R102G; the substitutions A113C, A120C, A339C, Q426C, T160F, I177L, Q100K, and S101A; the substitutions V84C, A140C, A147C, A249C, Q100R, and S101R; the substitutions A63C, A140C, A147C, K188C, K450C, S470C, Q100R, and S101R; the substitutions and deletion A63C, A140C, A147C, K188C, G106 deletion, Q100R, and S101R; the substitutions A113C, A120C, A339C, Q426C, T160F, I177L, Q100R, and S101R; the substitutions A185P, A113C, A339C, Q100R, and S101R; the substitutions A185P, T160F, I177L, Q100R, and S101R; the substitutions A185P, A113C, A339C, T160F, I177L, Q100R, and S101R; the substitutions A63C, K188C, N97G, P98G, R99G, Q100G, S101G, and R102G; the substitutions A63C, K188C, K450A, S470A, N97G, P98G, R99G, Q100G, S101G, and R102G; the substitutions A63C, A140C, A147C, K188C, G294E, N97G, P98G, R99G, Q100G, S101G, and R102G; the substitutions A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G, and G294E; the substitutions A63C, K188C, N97G, P98G, R99G, Q100G, S101G, R102G, and G294E; the substitutions T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, Q100R, and S101R; the substitutions A63C, A140C, A147C, K188C, K450C, S470C, N97G,

P98G, R99G, Q100G, S101G, and R102G; the substitutions V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, and R102G; the substitutions T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, N97G, P98G, R99G, Q100G, S101G, and R102G.

[0014] In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NOs: 58. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 59. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 60. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 61. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 62. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 63. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 64.

[0015] In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 65. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 66. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 67. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 68. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 69. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 70. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 71. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 72. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 73. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 74. In some embodiments, the hMPV F protein ectodomain comprises a sequence that

shares at least 95% or at least 99% identity to SEQ ID NO: 75. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 76. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 77. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 78. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 79. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 80. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 81. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 82. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 83. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 84. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 85. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 86. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 87. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 88. In some embodiments, the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 89. In some embodiments, the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 58, 60, 61, 77, 78, 86, 87, 88, or 89. In some embodiments, the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 59, 62, 63, 64, 71, 76, 81, or 83. In some embodiments, the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 72, 79, or 80. In some embodiments, the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 65, 66, 82, 84, 85, 90, or 91. In some embodiments, the hMPV F protein

ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 65, 79, or 80. In some embodiments, the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 92-124. In some embodiments, the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 97-116, 119, or 122. In some embodiments, the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 92-94, 96, 117, 118, 120, 121, 123, or 124. In some embodiments, the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 99, 112, 117, or 118.

[0016] In some embodiments, the VLP binds one or more hMPV F protein antibodies selected from MF1, MF2, MF3, MF9, MF12, MF14, MF15, MF11, MF16, MF20, MF17, MF18, MF19, MF10, or MPE8. In some embodiments, the VLP binds two or more antibodies selected from MF1, MF2, MF3, MF9, MF12, MF14, MF15, MF11, MF16, MF20, MF17, MF18, MF19, MF10, MPE8. In some embodiments, the VLP binds three or more antibodies selected from MF1, MF2, MF3, MF9, MF12, MF14, MF15, MF11, MF16, MF20, MF17, MF18, MF19, MF10, MPE8.

[0017] In some embodiments, the hMPV F protein ectodomain binds an antibody that preferentially binds the prefusion form of the hMPV F protein ectodomain. In some embodiments, the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 42, 45, 58, 61, 63, or 64. In some embodiments, the hMPV F protein ectodomain has no or low binding to an antibody that preferentially binds the postfusion form of the hMPV F protein ectodomain. In some embodiments, the VLP sequence shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 99, 115, 117, or 118.

[0018] In some embodiments, the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity with SEQ ID NO: 99.

[0019] In some embodiments, the first component is a single chain. In some embodiments, the single chain shares at least 85%, at least 90%, at least 95%, at least 99% or 100% identity with SEQ ID NO: 123.

[0020] In some embodiments, the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity with SEQ ID NO: 117.

[0021] In one aspect, the disclosure provides a polynucleotide encoding the VLP described herein.

[0022] In one aspect, the disclosure provides a host cell, comprising the polynucleotide described herein.

[0023] In one aspect, the disclosure provides a method of manufacturing a vaccine, comprising culturing the host cell described herein in a culture medium so that the host cell secretes the antigen into the culture media; optionally purifying the antigen from the culture media; mixing the antigen with a second component, wherein the second component multimerizes with the antigen to form a virus-like particle; and optionally purifying the virus-like particle.

[0024] In one aspect, the disclosure provides a vaccine, comprising the VLP described herein, wherein the vaccine optionally comprises one or more pharmaceutically acceptable diluents, adjuvants, or excipients. In some embodiments, the vaccine is a stable emulsion. In some embodiments, the vaccine comprises one or more adjuvants. In some embodiments, the one or more adjuvants is squalene, SLA, GLA, R848, IMQ, 3M-052, CpG, saponin (QS21), or combinations thereof. In some embodiments, the adjuvant is alum. In some embodiments, the adjuvant is a squalene-based emulsion. In some embodiments, the squalene-based emulsion is MF59.

[0025] In one aspect, the disclosure provides a method of immunizing a subject against infection by human metapneumovirus, the method comprising administering the vaccine described herein. In some embodiments, the subject is simultaneously immunized against infection by respiratory syncytial virus (RSV). In some embodiments, the vaccine is administered by subcutaneous injection. In some embodiments, wherein the vaccine is administered by intramuscular injection. In some embodiments, wherein the vaccine is administered by intradermal injection. In some embodiments, wherein the vaccine is administered intranasally. In one aspect, the disclosure provides a pre-filled syringe comprising the vaccine described herein. In one aspect, the disclosure provides a kit comprising the vaccine described herein or the pre-filled syringe described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] **FIG. 1A** shows an illustrative embodiment of a protein-based virus-like particle (VLP) according to the present disclosure.

[0027] **FIG. 1B** shows further illustrative embodiments of VLPs and VLP components (with G protein not shown).

[0028] **FIG. 2** shows a secondary structure map of hMPV F protein in the pre-fusion configuration (PDB ID: 5WB0; diagram generated at www.rcsb.org).

[0029] **FIGs. 3A-3C** is a series of plots showing antibody binding profiles to illustrative VLPs of the disclosure. Antibodies are specific for various forms of the hMPV F protein. MF14 binds pre- and postfusion forms of hMPV F protein at the site II epitope (**FIG. 3A**). MF16 binds pre- and postfusion forms of hMPV F protein at the site IV epitope (**FIG. 3B**). MPE8 binds the pre-fusion form of hMPV F protein at the site III epitope (**FIG. 3C**).

[0030] **FIG. 4** is a series of plots showing the time-course induction of neutralizing antibody titers in mice in response to dosing with hMPV F protein VLPs formulated with adjuvants.

[0031] **FIGs 5A and 5B** show the immunogenicity of hMPV F protein mutants on a 2-component virus-like particle (VLP). Graphs show neutralizing antibody titers against hMPV/A (**FIG. 5A**) and hMPV/B (**FIG. 5B**) in serum samples collected on day 35 after the first immunization.

[0032] **FIGs. 6A and 6B** show the immunogenicity of hMPV008 VLPs and the corresponding soluble protein, CompA-hMPV008. Graphs show neutralizing antibody titers against hMPV/A (**FIG. 6A**) and hMPV/B (**FIG. 6B**) in serum samples collected on day 35 after the first immunization.

[0033] **FIGs. 7A and 7B** show the immunogenicity of VLPs displaying an hMPV F protein mutant, hMPV008. Neutralizing antibody titers were determined for hMPV/A (**FIG. 7A**) and hMPV/B (**FIG. 7B**) in serum samples collected on day 35 after the first immunization.

[0034] **FIGs. 8A and 8B** show the immunogenicity of hMPV033. Neutralizing antibody titers were determined for hMPV/A (**FIG. 8A**) and hMPV/B (**FIG. 8B**) in serum samples collected on day 35 after the first immunization.

[0035] **FIGs. 9A-9C** show the protective effect of hMPV033 against hMPV infection in cotton rats. Neutralizing titers against hMPV/A were determined in serum (**FIG. 9C**), lung (**FIG. 9B**), and nasal (**FIG. 9C**) samples collected on day 35 after the first immunization.

DETAILED DESCRIPTION

[0036] Provided are protein-based virus-like particle (VLP) vaccines for human metapneumovirus (hMPV) in which the hMPV F protein ectodomain is linked to, and thereby displayed on, a protein-based VLP, *e.g.*, a designed VLP, *e.g.*, a symmetric VLP. For example, the vaccine antigen may be a N-terminal fusion of the ectodomain of hMPV F protein to a

protein having a multimerization domain for a one- or two-component *de novo* designed VLP, such as a two-component icosahedral VLP. Further provided are vaccine compositions, methods of manufacturing, and methods of use, *e.g.*, immunizing a subject to generate a protective immune response to hMPV virus.

Definitions

[0037] All publications, patents and patent applications, including any drawings and appendices therein are incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent or patent application, drawing, or appendix was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

[0038] The term “virus-like particle” or “VLP” refers to a molecular assembly that resembles a virus but is non-infectious that displays an antigenic protein, or antigenic fragment thereof, of a viral protein or glycoprotein. A “protein-based VLP” refers to a VLP formed from proteins or glycoproteins and substantially free of other components (*e.g.*, lipids). Protein-based VLPs may include post-translation modification and chemical modification, but are to be distinguished from micellar VLPs and VLPs formed by extraction of viral proteins from live or live inactivated virus preparations. The term “designed VLP” refers to a VLP comprising one or more polypeptides generated by computational protein design. Illustrative designed VLP are VLPs that comprise nanostructures depicted in FIG. 1B. The term “symmetric VLP” refers to a protein-based VLP with a symmetric core, such as shown in FIG. 1B. These include but are not limited to designed VLPs. For example, the protein ferritin has been used to generate a symmetric, protein-based VLP using naturally occurring ferritin sequences. Ferritin-based VLPs are distinguished from designed VLPs in that no protein engineering is necessary to form a symmetric VLP from ferritin, other than fusing the viral protein to the ferritin molecule. Protein design methods can be used to generate similar one- and two-component nanostructures based on template structures (*e.g.*, structures deposited in the Protein Data Bank) or *de novo* (*i.e.*, by computational design of new proteins having a desired structure but little or no homology to naturally occurring proteins). Such one- and two-component nanostructures can then be used as the core of a designed VLP.

[0039] The VLP of the present disclosure display antigens capable of eliciting immune responses to human metapneumovirus or other metapneumoviruses. The vaccines of the present disclosure are useful for preventing and/or decreasing the severity of infection with human metapneumovirus.

[0040] The term “icosahedral particle” refers to a designed VLP having a core with icosahedral symmetry (*e.g.*, the particles labeled I53 and I52 in **Table 1**). I53 refers to an icosahedral particle constructed from pentamers and trimers. I52 refers to an icosahedral particle constructed from pentamers and dimers. T33 refers to a tetrahedral particle constructed from two sets of trimers. T32 refers to a tetrahedral particle constructed from trimers and dimers.

[0041] The antigens may be attached to the core of the protein-based VLP either non-covalently or covalently, including as a fusion protein or by other means disclosed herein. Multimeric antigens may optionally be displayed along a symmetry axis of the VLP. Also provided are proteins and nucleic acid molecules encoding such proteins, formulations, and methods of use.

[0042] The term “antigen” refers to a polypeptide or polypeptide complex including at least one component designed to elicit an immune response. The term antigen, as used herein, is not limited to the portion of the polypeptide or polypeptide complex that contains antigenic epitopes.

[0043] The term “polypeptide” refers to a series of amino acid residues joined by peptide bonds and optionally one or more post-translational modifications (*e.g.*, glycosylation) and/or other modifications (including but not limited to conjugation of the polypeptide moiety used as a marker—such as a fluorescent tag—or an adjuvant).

[0044] The term “infection” refers to both symptomatic and asymptomatic infections.

[0045] The term “ectodomain” refers to the portion of a transmembrane protein or glycoprotein that, in the native state of the protein, is on the outside of the cellular or viral membrane.

[0046] The term “variant” refers to a polypeptide having one or more insertions, deletions, or amino acid substitutions relative to a reference polypeptide, but retains one or more properties of the reference protein.

[0047] The term “antigenic variant” refers to a variant that has one or more epitopes in common with a reference polypeptide and/or generates the same or similar immune response when administered to a subject as a reference polypeptide.

[0048] The term “functional variant” refers to a variant that exhibits the same or similar functional effect(s) as a reference polypeptide. For example, a functional variant of a multimerization domain is able to promote multimerization to the same extent, or to similar

extent, as a reference multimerization domain and/or is able to multimerize with the same cognate multimerization domains as a reference multimerization domain.

[0049] The term “linker” refers to either chemical linkage (*i.e.*, a covalent bond or series of covalent bonds with intervening chemical moieties) or to a polypeptide that is N-terminally and C-terminally joined by peptide bonds to product a fusion protein.

[0050] The term “domain” refers to any portion of a polypeptide that adopts a tertiary structure.

[0051] The terms “multimerization domain” and “multimerize” refer to the ability of a polypeptide, or domain of a polypeptide, to form dimers, trimers, tetramers, pentamers, or hexamers and/or to form heteromers with other multimerization domains.

[0052] The term “trimerization domain” refers to a multimerization domain that forms trimers.

[0053] The term “VLP-forming domain” refers to a multimerization domain that, alone or with other multimerization domains, forms a symmetric protein complex.

[0054] The term “fragment” refers to a polypeptide having one or more N-terminal or C-terminal truncations compared to a reference polypeptide.

[0055] The term “functional fragment” refers to a functional variant of a fragment.

[0056] The term “amino acid substitution” refers to replacing a single amino acid in a sequence with another amino acid residue. The standard form of abbreviations for amino acid substitution are used. For example, V94R refers to substitution of valine (V) in a reference sequence with arginine (R). The abbreviation Arg94 refers to any sequence in which the 94th residue, relative to a reference sequence, is arginine (Arg).

[0057] The terms “helix” or “helical” refer to an α -helical secondary structure in a polypeptide that is known to occur, or predicted to occur. For example, a sequence may be described as helical when computational modeling suggests the sequence is likely to adopt a helical conformation.

[0058] The term “component” refers to a protein, or protein complex, capable of assembly into a virus-like particle under appropriate conditions (*e.g.*, an antigen or polypeptide comprising a multimerization domain).

[0059] The term “vaccine” refers to a pharmaceutical composition capable of use in producing an immune response in a subject.

[0060] The term “pharmaceutically acceptable excipients” means excipients biologically or pharmacologically compatible for *in vivo* use in animals or humans, and can mean excipients

approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

[0061] The term “adjuvants” refers to a pharmaceutically acceptable substance that enhances the immune response to an antigen when co-administered with the antigen or administered before, during, or after administration of the antigen to a subject.

[0062] The term “TLR4 immunostimulant” refers to an adjuvant that stimulates Toll-like Receptor 4 (TLR4) in the immune cells of a subject to modulate an immune response *e.g.*, Monophosphoryl Lipid A (MPL), Glucopyranosyl Lipid A (GLA), and/or Soluble *Leishmania* Antigen (SLA).

[0063] The term “effective amount” refers to the amount of a formulation according to the invention that, when administered to a patient for treating a state, disorder or condition is sufficient to effect such treatment or when administered to a patient for generating an immune response is sufficient to generate such an immune response. The “effective amount” will vary depending on the active ingredient, the state, disorder, or condition to be treated and its severity, and the age, weight, physical condition and responsiveness of the subject to be treated.

[0064] The term “immune response” refers to elicitation of activity of one or more immune cell types in the subject. Immune responses include, for example, T cell and B cell responses.

[0065] The term “humoral immune response” refers to an immune response that generates plasma or serum antibodies (*e.g.*, IgG).

[0066] The term “protective immune response” refers to an immune response that prevents and/or reduces the severity of infection with a pathogen when the subject is later challenged with the pathogen, or to an immune response that generates a level of immune response that correlates with protection. For example, vaccination may generate a protective immune response if it results in production, in the plasma or serum, of the subject (*e.g.*, human, pet, or agricultural animal), of neutralizing antibodies that protect the subject against subsequent infection and/or are present in a quantity observed to confer protection upon test subjects (*e.g.*, New Zealand White (NZW) rabbits).

[0067] The term “polyclonal antibody response” refers to an antibody response comprising antibodies having more than one specificities and/or variation in their antibody sequences.

[0068] The term “neutralizing” (*e.g.*, “neutralizing antibody response”) refers to antibodies that prevent infection and/or reduce the level of infection by a pathogen. A neutralizing antibody response can be measured either in *in vitro* assays (*e.g.*, infection of cells in culture

by a pathogen in the presence of the antibody) or in an *in vivo* assay (*e.g.*, by determining a protective dose of an antibody through administering the antibody to a subject prior to challenge with an infective dose of a pathogen).

[0069] An antibody “binds to” or is “specific to” or “specifically binds” (used interchangeably herein) to a target (*e.g.*, human metapneumovirus or an hMPV protein) are terms well understood in the art, and methods to determine such specific or preferential binding are also well known in the art. A molecule is said to exhibit “specific binding” or “preferential binding” if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity with a particular cell or substance than it does with alternative cells or substances. For example, an immunoglobulin that specifically or preferentially binds to thymocyte is an immunoglobulin that binds thymocytes with greater affinity, avidity, more readily, and/or with greater duration than it binds to other cells. An immunoglobulin that specifically binds to a first cell or substance may or may not specifically or preferentially bind to a second cell or substance. As such, “specific binding” does not necessarily require (although it can include) exclusive binding. Generally, but not necessarily, reference to binding means specific binding.

[0070] The term “predetermined time” refers to an interval of time selected as appropriate for observing a particular effect. A predetermined time may be selected before, or during, an experiment or procedure.

[0071] The term “post-exposure prophylaxis” refers to administering an antigenic composition (*e.g.*, a vaccine) to a subject previously exposed to and/or infected with a pathogen in order to elicit an immune response to protect against infection by the pathogen and/or decrease the severity of one or more symptoms of infection by the pathogen.

[0072] The term “administering” refers to providing a composition to a subject in a manner that permits the composition to have its intended effect. Administration for vaccination or post-exposure prophylaxis may be performed by intramuscular injection, intravenous injection, intraperitoneal injection, or any other suitable route.

[0073] The terms “immunization” and “immunizing” refer to administering a composition to a subject (*e.g.*, a virus-like particle) in an amount sufficient to elicit, after one or more administering steps, a desired immune response (*e.g.*, a humoral immune response to the VLP). Immunization may comprise between one and ten, or more administrations (*e.g.*, injections) of the composition, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more administrations. The first administration may elicit no detectable immune response as generally each subsequent

administration will boost the immune response generated by prior administrations. The term “immunizing” as used herein includes post-exposure prophylaxis.

[0074] The term “subject” refers to a human or non-human animal to which a composition may be administered for vaccination, treatment, or other purpose. In some embodiments, the non-human animal is a non-human primate, rabbit, hamster, gerbil, pig, cow, sheep, goat, guinea pig, rat, mouse, squirrel, wolf, fox, horse, zebra, giraffe, elephant, cat, dog, llama, or ferret.

[0075] The term “manufacturing” refers to production of a recombinant polypeptide or virus-like particle at any scale, including at least 25-mL, 50-mL, 1-L, 1,000-L, 50,000-L, or greater scale.

[0076] The terms “culturing” and “culture medium” refers to standard cell culture and recombinant protein expression techniques.

[0077] The term “host cell” refers to any cell capable of use in expression of a recombinant polypeptide.

[0078] The term “secretes” refers to the ability of host cells to secrete polypeptides into the media in which they are cultured.

[0079] The term “signal sequence” refers to a polypeptide sequence, typically at the N terminus of a polypeptide expressed in a host cell, that directs the polypeptide to a particular cellular compartment. A signal sequence may be a secretion signal to cause the host cell to secrete the polypeptide into the media in which with host cell is cultured. Various signal sequences are known and it is within the skill of an ordinary artisan to select an appropriate signal sequence.

[0080] The term “mixing” refers to placing two solutions into contact to permit the solutions to mix.

[0081] The term “purify” refers to separating a molecule from other substances present in a composition. Polypeptides may be purified by affinity (*e.g.*, to an antibody or to a tag, *e.g.*, using a His-tag capture resin), by charge (*e.g.*, ion-exchange chromatography), by size (*e.g.*, preparative ultracentrifugation, size exclusion chromatography), or otherwise.

[0082] The terms “polynucleotide” and “nucleic acid,” used interchangeably herein, refer to a polymeric form of nucleotides of more than about 100 nucleotides, either ribonucleotides or deoxyribonucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically

modified, non-natural, or derivatized nucleotide bases. “Oligonucleotide” generally refers to polynucleotides of between about 5 and about 100 nucleotides of single- or double-stranded DNA. However, for the purposes of this disclosure, there is no upper limit to the length of an oligonucleotide. Oligonucleotides are also known as “oligomers” or “oligos” and may be isolated from genes, or chemically synthesized by methods known in the art. The terms “polynucleotide” and “nucleic acid” should be understood to include, as applicable to the embodiments being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides.

[0083] The terms “identity”, “identical”, and “sequence identity” refer to the percentage of bases or amino acids between two polynucleotide or polypeptide sequences that are the same, and in the same relative position. As such one polynucleotide or polypeptide sequence has a certain percentage of sequence identity compared to another polynucleotide or polypeptide sequence. For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. The term “reference sequence” refers to a molecule to which a test sequence is compared.

[0084] Methods of sequence alignment for comparison and determination of percent sequence identity is well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the homology alignment algorithm of Needleman and Wunsch, (1970) *J. Mol. Biol.* 48:443, by the search for similarity method of Pearson and Lipman, (1988) *Proc. Nat'l. Acad. Sci. USA* 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), by manual alignment and visual inspection (see, *e.g.*, Brent *et al.*, (2003) *Current Protocols in Molecular Biology*), by use of algorithms known in the art including the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, (1977) *Nuc. Acids Res.* 25:3389-3402; and Altschul *et al.*, (1990) *J. Mol. Biol.* 215:403-410, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

[0085] The term “treating” means one or more of relieving, alleviating, delaying, reducing, reversing, improving, or managing at least one symptom of a condition in a subject. The term “treating” may also mean one or more of arresting, delaying the onset (*i.e.*, the period prior to clinical manifestation of the condition) or reducing the risk of developing or worsening a condition.

[0086] The term “about” or “approximately” means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, *e.g.*, the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation. Alternatively, “about” can mean plus or minus a range of up to 20%, up to 10%, or up to 5%.

[0087] All weight percentages (*i.e.*, “% by weight” and “wt. %” and w/w) referenced herein, unless otherwise indicated, are measured relative to the total weight of the pharmaceutical composition.

[0088] As used herein, “substantially” or “substantial” refers to the complete or nearly complete extent or degree of an action, characteristic, property, state, structure, item, or result. For example, an object that is “substantially” enclosed would mean that the object is either completely enclosed or nearly completely enclosed. The exact allowable degree of deviation from absolute completeness may in some cases depend on the specific context. However, generally speaking, the nearness of completion will be so as to have the same overall result as if absolute and total completion were obtained. The use of “substantially” is equally applicable when used in a negative connotation to refer to the complete or near complete lack of action, characteristic, property, state, structure, item, or result. For example, a composition that is “substantially free of” other active agents would either completely lack other active agents, or so nearly completely lack other active agents that the effect would be the same as if it completely lacked other active agents. In other words, a composition that is “substantially free of” an ingredient or element or another active agent may still contain such an item as long as there is no measurable effect thereof.

[0089] The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed inventions, or that any publication specifically or implicitly referenced is prior art.

Overview

[0090] Vaccination is a treatment modality used to prevent or decrease the severity of infection with various infectious agents, including bacteria, viruses, and parasites. Development of new vaccines has important commercial and public health implications. In particular, lyme disease, pertussis, herpes virus, orthomyxovirus, paramyxovirus, pneumovirus, filovirus, flavivirus, reovirus, retrovirus, coronavirus, and malaria are infectious agents for which vaccines already exist, are being developed, or would be desirable.

[0091] Subunit vaccines are vaccines made from isolated antigens, usually proteins expressed recombinantly in bacterial, insect, or mammalian cell hosts. Typically, the antigenic component of a subunit vaccine is selected from among the proteins of an infectious agent observed to elicit a natural immune response upon infection, although in some cases other components of the infectious agent can be used. Typical antigens for use in subunit vaccines include protein expressed on the surface of the target infectious agent, as such surface-expressed envelope glycoproteins of viruses. Preferably, the antigen is a target for neutralizing antibodies. More preferably, the antigen is a target for broadly neutralizing antibodies, such that the immune response to the antigen covers immunity against multiple strains of the infectious agent. In some cases, glycans that are N-linked or O-linked to the subunit vaccine may also be important in vaccination, either by contributing to the epitope of the antigen or by guiding the immune response to particular epitopes on the antigen by steric hindrance. The immune response that occurs in response to vaccination may be direct to the protein itself, to the glycan, or to both the protein and linked glycans. Subunit vaccines have various advantages including that they contain no live pathogen, which eliminates concerns about infection of the patient by the vaccine; they may be designed using standard genetic engineering techniques; they are more homogenous than other forms of vaccine; and they can be manufactured in standardized recombinant protein expression production systems using well-characterized expression systems. In some cases, the antigen may be genetically engineered to favor generation of desirable antibodies, such as neutralizing or broadly neutralizing antibodies. In particular, structural information about an antigen of interest, obtained by X-ray crystallography, electron microscopy, or nuclear magnetic resonance experiments, can be used to guide rational design of subunit vaccines.

[0092] A known limitation of subunit vaccines is that the immune response elicited may sometimes be weaker than the immune response to other types of vaccines, such as whole virus, live, or live-attenuated vaccines. The present inventors have recognized and herein disclose that designed and/or protein-based VLP vaccines have the potential to harness the advantages of subunit vaccines while increasing the potency and breadth of the vaccine-induced immune response through multivalent display of the antigen in symmetrically ordered arrays. In the present disclosure, protein-based VLPs are distinguished from nanoparticle vaccines, because the term nanoparticle vaccine has been used in the art to refer to protein-based or glycoprotein-based vaccines (*see, e.g.* Patent No. US 9,441,019), polymerized liposomes (*see, e.g.*, US

Patent No. 7,285,289), surfactant micelles (*see, e.g.*, US Patent Pub. No. US 2004/0038406 A1), and synthetic biodegradable particles (*see, e.g.*, US Patent No. US 8,323,696).

[0093] The protein-based VLPs of the present disclosure are distinguishable from VLPs that display an antigen on the surface of a micelle particle containing a surfactant (*e.g.*, NP-9); or alternatively, made by extracting antigenic proteins from live virus while retaining lipid constituents of the viral envelope. By contrast, the protein-based VLPs described herein are free of, or substantially free of, lipid and surfactants. Furthermore, the symmetric display of the hMPV F protein on some embodiments of the protein-based VLPs of this disclosure may generate superior immune response to the F protein compared to other VLPs.

Embodiments

[0094] The present disclosure relates to protein-based VLPs and protein-based VLP vaccines. The VLP of the present disclosure display an antigen capable of eliciting immune responses to hMPV. Some vaccines of the present disclosure are useful for preventing or decreasing the severity of infection with hMPV. In particular, the antigens of the disclosure display the ectodomain of the hMPV F protein. The ectodomain may be attached to the core of the VLP either non-covalently or covalently, including as a fusion protein or by other means disclosed herein. In some embodiments, a linker connects the ectodomain to a first polypeptide comprising a multimerization domain. The linker may be any chemical linkage including but not limited to a polypeptide used to form a N-terminal or C-terminal fusion of the ectodomain to the first polypeptide. The hMPV F protein may optionally be displayed along a symmetry axis of the VLP. In some embodiments, the F protein is C-terminally linked to the first polypeptide comprising a trimerization domain and optionally an additional trimerization tag (*e.g.*, FoldOn tag). Also provided are proteins and nucleic acid molecules encoding such proteins, formulations, and methods of use.

Protein-based Virus-Like Particles

[0095] The VLPs of the present invention may comprise multimeric protein assemblies adapted for display of the ectodomain of hMPV F protein, or an antigenic fragment thereof. The VLPs of the present invention comprise at least a first plurality of polypeptides. The first plurality of polypeptides (also referred to a “first component”) may be derived from a naturally-occurring protein sequence by substitution of at least one amino acid residue or by additional at the N- or C-terminus of one or more residues. In some cases, the first component comprises

a protein sequence determined by computational methods. This first component may form the entire core of the VLP; or the core of the VLP may comprise one or more additional polypeptides (also referred to a “second component” or third, fourth, fifth component and so on), such that the VLP comprises two, three, four, five, six, seven, or more pluralities of polypeptides. In some cases, the first plurality will form trimers related by 3-fold rotational symmetry and the second plurality will form pentamers related by 5-fold rotational symmetry. In such cases, the VLP forms an “icosahedral particle” having I₅₃ symmetry. Together these one or more pluralities of polypeptides may be arranged such that the members of each plurality of polypeptides are related to one another by symmetry operators. A general computational method for designing self-assembling protein materials, involving symmetrical docking of protein building blocks in a target symmetric architecture, is disclosed in Patent Pub. No. US 2015/0356240 A1.

[0096] The “core” of the VLP is used herein to describe the central portion of the VLP that links together the several copies of the hMPV F protein ectodomain, or antigenic fragments thereof, displayed by the VLP. In an embodiment, the first component comprises a first polypeptide comprising a hMPV F protein ectodomain or antigenic variant thereof, a linker, and a first polypeptide comprising a multimerization domain. Thereby, the “antigen” is a single polypeptide, capable of self-assembly into a VLP either alone or this a second component (third, fourth, or fifth, etc., components). An advantage of designing the antigens themselves to self-assemble is that the entire VLP then acts as the antigenic component of the vaccine. In other embodiments, the ectodomain, or antigenic fragment thereof, is non-covalently or covalently linked to the first polypeptide comprising a multimerization domain. For example, an antibody or antigenic fragment thereof may be fused to the first polypeptide and configured to bind a portion of the first polypeptide, or a chemical tag on the first polypeptide. A streptavidin-biotin (or neuravidin-biotin) linker can be employed. Or various chemical linkers may be used. An advantage of designing a core to be a generic platform is that the one or more pluralities of polypeptides that comprise the core can be designed and optimized in advance and then applied to the ectodomain serially to identify VLP(s) having superior efficacy as vaccines. It will be understood that in some cases, the same polypeptide may form a portion of the “core” and then extend outward as either an adaptor for attachment of the hMPV F protein ectodomain and to include the ectodomain (*i.e.*, a fusion protein). In embodiments of the present disclosure, the antigen comprises further polypeptide sequences in addition to the hMPV F protein ectodomain. In certain embodiments, the ectodomain is glycosylated either

natively or using alternative oligosaccharides (*e.g.*, oligosaccharides specific to the host cell used to express the antigen).

[0097] In some cases, self-assembly may be further promoted by multimerization of the ectodomain even though the core would, in absence of the ectodomain, be independently capable of self-assembly. The hMPV F protein is detected as a trimer in its native state (Cseke et al. *J. Virol.* 21(2):698-707 (2007)). Display of the ectodomain on a VLP, at least in some embodiments, decreases the thermodynamic barrier for assembly and/or the equilibrium ratio of assembled to non-assembled VLP components. In some cases, the trimeric ectodomain placed along a 3-fold axis of the VLP promotes proper folding and conformation stability of the ectodomain and makes self-assembly of the VLP a cooperative process, in that the ectodomain is trimerized properly in part due to its display on a 3-fold axis of the core of the VLP, and the VLP is stabilized in its assembled form, at least in part, by non-covalent or covalent interactions amongst the trimer units. In some cases, introduction of mutations to the antigen or to the VLP components may optionally further stabilize assembly, in particular if cysteine residues are positioned to create intramolecular disulfide bonds. In some examples, a dimeric, trimeric, tetrameric, pentameric, or hexameric antigen is displayed upon a core designed to have a matching 2-fold, 3-fold, 4-fold, 5-fold, or 6-fold symmetry axis such that the core accommodates the arrangement of the multimeric antigen with the native symmetry of the antigen.

Various Non-Limiting Examples of VLPs

[0098] A non-limiting example of an embodiment is shown in **FIG. 1A**, which depicts an ectodomain antigen genetically fused to a component (a first plurality of polypeptides) of the VLP, which optionally is expressed recombinantly in a host cell (*e.g.*, 293F cells); along with a pentameric protein assembly (a second plurality of polypeptides), which is optionally expressed recombinantly in the same or a different host cell (*e.g.*, *E. coli* cells), these two pluralities of polypeptides self-assembling into a VLP displaying 20 antigen trimers around an icosahedral core. In this embodiment, the core has a generic design. As explained below, in other embodiments, an antigen of the disclosure is mixed with another antigen protein in the same VLP, such as an ectodomain of a protein from a second virus, or such as a trimeric glycoprotein from another virus. In some embodiments, the VLP comprises, in addition to a hMPV F protein ectodomain, the trimeric glycoproteins of HIV-1, HIV-2, EBV, CMV, RSV, influenza, Ebola, Marburg, Dengue, SARS, MERS, Hanta, or Zika virus. In some

embodiments, the VLP comprises the trimeric glycoproteins of viruses that are related evolutionarily or in sequence identity to any of these illustrative virus, including without limitation, a herpes virus, orthomyxovirus, paramyxovirus, pneumovirus, filovirus, flavivirus, reovirus, or retrovirus. In an embodiment, the VLP comprises the extracellular domain or domains of a transmembrane protein or glycoprotein, or an antigenic fragment thereof. In some embodiments, the VLP is further linked to polypeptides or other agents capable of acting as an adjuvant. In some embodiments, the antigen (first component) and/or the second component comprises one or more T cell epitopes, optionally a T cell epitope of heterologous origin.

[0099] Trimeric antigens that may be used with VLPs of the disclosure to form mixed VLPs are in some cases, without limitation, SARS-CoV-2, respiratory syncytial virus, HIV gp140, influenza HA, dengue E protein, or Ebola sGP. Mixed VLPs of the disclosure include those that comprise both a hMPV F protein ectodomain and antigenic protein(s) from one or more of Vesicular stomatitis virus, Herpes simplex virus, Baculovirus, Thogotovirus, and Bornaviridae. When other trimeric antigens are used, they may optionally be placed on the 3-fold symmetry axis of the VLP. In some cases, the antigen chosen is monomeric and nevertheless placed on a 3-fold axis. Thus, the VLP depicted in **FIG. 1A** is capable of displaying 20 trimeric antigens or 60 monomeric antigens. Additionally or alternatively the pentameric complexes of the VLP is used to display a 12 pentameric antigens or 70 monomeric antigens. In an embodiment, the VLP comprises 20 copies of a trimeric antigen and 12 copies of a pentameric antigen.

VLP Cores

[0100] Other potential arrangements of polypeptides of the present disclosure are shown in **FIG. 1B**. In some embodiments, the VLP is adapted for display of up to 8 trimers; 8 trimers and 12 dimers; 6 tetramers and 12 dimers; 6 tetramers and 8 trimers; 20 trimers and 30 dimers; 4 trimers and 6 dimers; 4 first trimers and 4 second trimers, or 8 trimers; 12 pentamers and 20 trimers; or 12 pentamers and 30 dimers; or 4 trimers. In some cases, one of the symmetric axes is not used for antigen display, thus, in some embodiments the VLP is adapted for display of up to 8 trimers; 12 dimers; 6 tetramers; 20 trimers; 30 dimers; 4 trimers; 6 dimers; 8 trimers; or 12 pentamers. In some cases, monomeric antigens are displayed and thus, the VLP is adapted for display of up to 12, 24, 60, or 70 monomeric antigens. In some cases, the VLP comprises mixed pluralities of polypeptides such that otherwise identical polypeptides of the core of the VLP display different antigens or no antigen. Thus, depending on the ratio of polypeptides, the VLP is in some cases adapted for display of between 1 and 130 antigens (*e.g.*, on the I52 particle) where each of the antigens displayed may be the same or may be different members

of mixed population in proportion to any ratio chosen. The antigens may be co-expressed in a recombinant expression system and self-assembled before purification. Alternatively, the antigens may be expressed separately and then mixed together, either before or after purification from expression host and associated contaminants. In various embodiments, 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, or more antigens are displayed. Non-limiting illustrative VLPs are provided in Bale et al. *Science* 353:389-94 (2016); Heinze et al. *J. Phys. Chem B.* 120:5945-5952 (2016); King et al. *Nature* 510:103-108 (2014); and King et al. *Science* 336:1171-71 (2012).

Mixed VLPs

[0101] In some cases, the VLP is adapted to display the same antigen from two or more diverse strains of hMPV. In non-limiting examples, the same VLP displays mixed populations of homotrimeric protein antigens or mixed heterotrimers of protein antigens from different strains of hMPV. In an embodiment, the VLP displays the F proteins, or antigenic fragment thereof, disclosed in any sequence of GenBank found by searching the Protein database with the keyword “human metapneumovirus F,” individually or in mixed VLPs.

[0102] When mixed VLPs are made, it may be advantageous to ensure formation of homomultimers in a strain-specific manner rather than permit heteromultimerization, such that, for example all strain 1 F proteins are displayed on one 3-fold axis of a T33 particle whereas all strain 2 F proteins are displayed on the other 3-fold axis of the T33 particle. This may be achieved by use a VLP comprising two or more pluralities of polypeptides as the core of the VLP with each plurality of polypeptides attached to a different antigen. Alternatively, a VLP may be engineered with one or more symmetry-breaking mutations, such as knob-in-hole mutations or intramolecular disulfide mutations, which have the effect of preventing trimer formation between the different antigens. In that case, the VLP displays multimeric antigens from different strains at symmetrically equivalent positions on the VLP, but each position on the VLP is occupied by homomultimers from the same strain, with only an insignificant proportion of inter-strain heteromeric antigens. In some cases, the antigen itself may be genetically engineered to prevent inter-strain heteromultimerization. In an embodiment, the VLP is engineered to prevent heteromultimerization of two antigenic proteins with conserved structure but divergent antigenicity, such as for example, a strain 1 F protein and strain 2 F protein, or a hMPV F protein and a non-hMPV antigenic protein. Furthermore, when mixed VLPs are made and the antigens are displayed as fusion proteins, the VLP will comprise three or more different proteins, as the fusion proteins will share identical (or equivalent) domains

used to form the core of the VLP with different antigenic domains, one for each antigen displayed on the VLP.

Attachment Modalities (Linkers)

[0103] The VLPs of the present disclosure display antigenic proteins in various ways including as gene fusion or by other means disclosed herein. As used herein, “linked to” or “attached to” denotes any means known in the art for causing two polypeptides to associate. The association may be direct or indirect, reversible or irreversible, weak or strong, covalent or non-covalent, and selective or nonselective.

[0104] In some embodiments, attachment is achieved by genetic engineering to create an N- or C-terminus fusion of an antigen to one of the pluralities of polypeptides composing the VLP. Thus, the VLP may consist of, or consist essentially of, one, two, three, four, five, six, seven, eight, nine, or ten pluralities of polypeptides displaying one, two, three, four, five, six, seven, eight, nine, or ten pluralities of antigens, where at least one of the pluralities of antigen is genetically fused to at least one of the plurality of polypeptides. In some cases, the VLP consists essentially of one plurality of polypeptides capable of self-assembly and comprising the plurality of antigenic proteins genetically fused thereto. In some cases, the VLP consists essentially of a first plurality of polypeptides comprising a plurality of antigens; and a second plurality of polypeptides capable of co-assembling into two-component VLP, one plurality of polypeptides linking the antigenic protein to the VLP and the other plurality of polypeptides promoting self-assembly of the VLP.

[0105] In some embodiments, attachment is achieved by post-translational covalent attachment between one or more pluralities of polypeptides and one or more pluralities of antigenic protein. In some cases, chemical cross-linking is used to non-specifically attach the antigen to a VLP polypeptide. In some cases, chemical cross-linking is used to specifically attach the antigenic protein to a VLP polypeptide (*e.g.* to the first polypeptide or the second polypeptide). Various specific and non-specific cross-linking chemistries are known in the art, such as Click chemistry and other methods. In general, any cross-linking chemistry used to link two proteins may be adapted for use in the presently disclosed VLPs. In particular, chemistries used in creation of immunoconjugates or antibody drug conjugates may be used. In some cases, an VLP is created using a cleavable or non-cleavable linker. Processes and methods for conjugation of antigens to carriers are provided by, *e.g.*, Patent Pub. No. US 2008/0145373 A1.

[0106] In an embodiment, attachment is achieved by non-covalent attachment between one or more pluralities of polypeptides and one or more pluralities of antigen. In some cases, the

antigenic protein is engineered to be negatively charged on at least one surface and the core polypeptide is engineered to be positively charged on at least one surface, or positively and negatively charged, respectively. This promotes intermolecular association between the antigenic protein and the core polypeptide by electrostatic force. In some cases, shape complementarity is employed to cause linkage of antigen protein to core. Shape complementarity can be pre-existing or rationally designed. In some cases, computational designed of protein-protein interfaces is used to achieve attachment. In an embodiment, the antigen is biotin-labeled and the polypeptide comprises a streptavidin, or vice versa. In an embodiment, streptavidin is displayed by gene fusion or otherwise as a tetramer on a 4-fold axis of the core and the biotin-labeled antigen is monomeric, dimeric, or tetrameric, permitting association to the core in a configuration appropriate for native multimerization of the antigen. In some cases, a protein-based adaptor is used to capture the antigenic protein. In some cases, the polypeptide is fused to a protein capable of binding a complementary protein, which is fused to the antigenic protein.

[0107] The immune reaction to hMPV F may be controlled by altering the orientation of the ectodomain relative to the core. Depending on how the antigenic protein is attached to the core of the VLP, the antigenic protein may be displayed in various orientations. In some embodiments, the antigenic protein is displayed so that one or more known epitopes are oriented at or towards the distal end of the antigenic protein, such that these epitope(s) are preferentially accessible to the immune system. In some cases, the orientation will recapitulate the orientation of a viral protein with respect to the virus. The choice of orientation may direct the immune system to one or the other epitope.

[0108] In some embodiments, epitope preference is control by other means, such as positioning of glycans on the VLP by addition or subtraction of the N-linked glycan sequence motif N-X-[T/S] at predetermined positions in the amino acid sequence of any of the polypeptides of the VLP including in the amino acid sequence of the antigenic protein.

[0109] In some cases, the epitopes found at intermediate distances from the proximal to the distal end will be the preferred over epitopes more distally located depending on various considerations including but not limited to the overall geometry of the VLP, surface hydrophobicity, surface charge, and competitive binding of proteins endogenously present in the subject or proteins exogenously provided in the vaccine composition. The present disclosure encompasses all known methods of rational design of protein structure and the foregoing is not intended to be limiting.

VLP Polypeptide Sequences

[0110] The polypeptides of the present disclosure may have any of various amino acids sequences. Patent Pub No. US 2015/0356240 A1 describes various methods for designing protein assemblies. As described in US Patent Pub No. US 2016/0122392 A1 and in International Patent Pub. No. WO 2014/124301 A1, the isolated polypeptides of SEQ ID NOS:1-51 were designed for their ability to self-assemble in pairs to form VLPs, such as icosahedral particles. The design involved design of suitable interface residues for each member of the polypeptide pair that can be assembled to form the VLP. The VLPs so formed include symmetrically repeated, non-natural, non-covalent polypeptide-polypeptide interfaces that orient a first assembly and a second assembly into a VLP, such as one with an icosahedral symmetry. Thus, in one embodiment the first polypeptide and second polypeptides (that is, the two polypeptides of the core of the VLP) are selected from the group consisting of SEQ ID NOS:1-51. In each case, an N-terminal methionine residue present in the full length protein but may be removed to make a fusion is not included in the sequence. The identified residues in Table 1 are numbered beginning with an N-terminal methionine (not shown). In various embodiments, one or more additional residues are deleted from the N-terminus and/or additional residues are added to the N-terminus (*e.g.* to form a helical extension).

TABLE 1

Name	Component Multimer	Amino Acid Sequence	Identified interface residues
I53-34A SEQ ID NO:1	trimer	EGMDPLAVLAESRLLPLLTVRGGEDLAGLATVLELMGVGALEITLRTEKGLE ALKALRKSGLLLGAGTVRSPEAEAALEAGAAFLVSPGLLEEVAALAAQARGV PYLPGLVTPTEVERALALGLSALKFFPAEPFQGVRLRAYAEVFPVRFPLPT GGIKEEHLPHYAALPNLLAVGGSWLLQGDLAAMKVKVKAALKLLSPQAPG	I53-34A: 28, 32, 36, 37, 186, 188, 191, 192, 195
I53-34B SEQ ID NO:2	pentamer	TKKVGIVDTTTFARVDMASAAIRTLKALSFNKIIRKTVPGIKDLPVACKLL EEEGCDIVMALGMPGKAEKDKVCAHEASLGLMLAQLMTNKHIIIEVFNHEDEA KDDDELIDILALVRAIEHAANVYLLFKPEYLTRMAGKGLRQGREDA GPARE	I53-34B: 19, 20, 23, 24, 27, 109, 113, 1 16, 117, 120, 1 24, 148
I53-40A SEQ ID NO:3	pentamer	TKKVGIVDTTTFARVDMASAAITLKMESFNKIIRKTVPGIKDLPVACKLL EEEGCDIVMALGMPGKAEKDKVCAHEASLGLMLAQLMTNKHIIIEVFNHEDEA KDDAELKILAAARRAIEHALNVYLLFKPEYLTRMAGKGLRQGFEDAGPARE	I53-40A: 20, 23, 24, 27, 28, 109, 112, 1 13, 116, 120, 1 24
I53-40B SEQ ID NO:4	trimer	STINNQLKALKVIPVIAIDNAEDIIPLGKVLAEGLPAEITFRSSAAVKAI MLLSAQPEMLIGAGTIILNGVQALAAKEAGATFVSPGFNPNTVRACQIIIGI DIVPGVNNPSTVEAALEMGLTTLKFFPAEASGGISMVKSILVGPYGDIRLMPT GGITPSNIDNYLAIPQVLACGGTWMVDKLVNNGEWDEIARLTREIVEQVNP	I53-40B: 47, 51, 54, 58, 74, 102
I53-47A SEQ ID NO:5	trimer	PIFTLNTNIKATDVPDSDFLSLTSRLVGLILSKPGSYVAVHINTDQQLSFGGS TNPAAFGLTMSIGGIEPSKNRDHSAVLFDHLNAMLGIPKNRMYIHFVNLDG DVGWNGTTF	I53-47A: 22, 25, 29, 72, 79, 86, 87
I53-47B SEQ ID NO:6	pentamer	NQSHKDYETVRIAVVRARWHADIVDACVEAFEIAMAAGGDRFAVDVFDVP GAYETPLHARTLAETGRYGAVLGTAFVNVNGGIYRHEFVASAVIDGMMNVQLS TGVPVLSAVLTPHRYRDSAEHHRFFAAHFAVKGVEAARACIEILAAREKIAA	I53-47B: 28, 31, 35, 36, 39, 131, 132, 1 35, 139, 146

Name	Component Multimer	Amino Acid Sequence	Identified interface residues
I53-50A SEQ ID NO:7	trimer	EELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIETFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTLELVKAMKLGHTILKLPFGEVVGPPQFVKAMKGPFPNVKFPVPTGGVNLDNVCEWFKAGVLAAGVGSALVKGTPDEVREKAKAFVEKIRGCTE	I53-50A: 25, 29, 33, 54, 57
I53-50B SEQ ID NO:8	pentamer	NQHSKDYETVRIAVVRARWHAEIVDACVSFAEAMADIGGDRFAVDVFDVPGAYEIPHLARTLAETGRYGAVLGTAFVVGNGIYRHEFVASAVIDGMNVQLSTGVFVLSAVLTPHRYRSDAHTLLFLALFAVKGMEAAARACVEILAAREKIAA	I53-50B: 24, 28, 36, 124, 125, 127, 128, 129, 131, 132, 133, 135, 139
I53-51A SEQ ID NO:9	trimer	FTKSGDDGNTNVINKRVGKDSPLVNFGLDDELNSFIGFAISKIPWEDMKKDLERVQVLEFIEGEDLSTQSSKKKIDESVYVWLLAATAIYRIESGPKLVFIPGGSEASVLHVTRSVARVERNAVYTKELPEINRMIIYVNLRLSSLLFAMALVANKRRNQSEKIYEIGKSW	I53-51A: 80, 83, 86, 87, 88, 90, 91, 94, 166, 172, 176
I53-51B SEQ ID NO:10	pentamer	NQHSKDYETVRIAVVRARWHADIVDQCVRAFEEMADAGGDRFAVDVFDVPGAYEIPHLARTLAETGRYGAVLGTAFVVGNGIYRHEFVASAVIDGMNVQLSTGVFVLSAVLTPHRYRSSREHHEFFREHFVMKGVAAAAACITILAAREKIAA	I53-51B: 31, 35, 36, 40, 122, 124, 128, 131, 135, 139, 143, 146, 147
I52-03A SEQ ID NO:11	pentamer	GHTKGPTPQQHDGGSALRIGIVHARWNKTIIMPLLIIGTIAKLLCEGVKASNIVVQSVPGSWELPIAVQRLYSASQLQTPSSGSPLSAGDLLGSSTDLTALPTTTASSTGPFDAIAGVLIKGETMHFEYIADSVSHGLMRVQLDTGVVPVIFGVLTVLTDDQAKARAGVIEGSHNHGEDWGLAAVEMGVRRRDWAAGKTE	I52-03A: 28, 32, 36, 39, 44, 49
I52-03B SEQ ID NO:12	dimer	YEVHDADVYDLFYLGKGRKDYAAEASDIADLVRSRTPEASSLLDVACGTGTHLEHFTKEFGDTAGLESEDMLTTHARKLPDATHLQGDMDRDFQLGRKFSAVVSMFSSVGYLKTVAELGAAVASFAEHLEPGGVVVVEPWWFETFADGWVSADVVRRDGRTVARVSHSVREGNATRMVHFTVADPGKGVRRHFSVHLITLTFHQREYEAAAFMAAGLRVEYLEGGPSGRGLFVGVPAA	I52-03B: 94, 115, 116, 206, 213
I52-32A SEQ ID NO:13	dimer	GMKEKFLVLIITHGDFGKGLLSGAEVIIGKQENVHTVGLNLGDNIEKVAKEVMRIIIAKLAEDKEIIIVVDLFGGSPFNIALEMMKTFDVKVITGINMFMPLVELLTSINVYDTTELLENISKIGKDGKIVIEKSSSLKM	I52-32A: 47, 49, 53, 54, 57, 58, 61, 83, 87, 88
I52-32B SEQ ID NO:14	pentamer	KYDGSKLRIGILHARWNLETTAALVAGAIKRLQEFVKAENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIPIGVLIKGSTMHFEYICDSTTHQLMKLNFELGIPVIFGVLTCLTDEQAARAGLIEGKMHNHGEDWGAAAVEMATKFN	I52-32B: 19, 20, 23, 30, 40
I52-33A SEQ ID NO:15	pentamer	AVKGLGEVDQKYDGSKLRIGILHARWNRIILALVAGAVLRLLLEFGVKAENIIETVPGSFELPYGSKLFVEKQKRLGKPLDAIPIGVLIKGSTMHFEYICDSTTHQLMKLNFELGIPVIFGVLTCLTDEQAARAGLIEGKMHNHGEDWGAAAVEMATKFN	I52-33A: 33, 41, 44, 50
I52-33B SEQ ID NO:16	dimer	GANWYLDNESSRLSFTSTKNADIAEVHRFLVLHGKVDPKGLAEVEVETESIS TGIPLRDMLLRVLVQVSKFPVQAQINAQLDMRPINNLPAGQLELRLPLTVSLRGKSHSYNAELLATRLDERRFQVVTLEPLVHAQDFDMVRAFNALRLVAGLSAVSLSVFVGAVLI FTAR	I52-33B: 61, 63, 66, 67, 72, 147, 148, 154, 155
I32-06A SEQ ID NO:17	dimer	TDYIRDGSAIKALSFAITLAEADLRHIPQDLQRLAVRVIHACGMVDVANDLAFSEGAGKAGRNALLAGAPILCDARMVAEGITRSRLPADNRVIYTLSDPSVPELAKKIGNTRSAALDLWLPHITEGSIIVAGNAPTALFRLELFDAGAPKPAIIGMPVGVFGAAESKDELAANSRGVPYVIVRGRGGSAMTAAAVNALASERE	I32-06A: 9, 12, 13, 14, 20, 30, 33, 34
I32-06B SEQ ID NO:18	trimer	ITVFGLKSKLAPRREKLAETVYSSHLGLDIPKQKHAIKFLCLEKEDFYYPFDRSDDYTVIEINLMAGRSEETKMLLIFLLFIALERKLGIRAHVDVEITIKEQPAHCWGFRGRTGDSARDLDYDIYV	I32-06B: 24, 71, 73, 76, 77, 80, 81, 84, 85, 88, 114, 118
I32-19A SEQ ID NO:19	trimer	GSDLQKLQRFSTCDISDGLLVNVIPTGGYFPNLTAISPPQNSSIVGTAYTVLFAPIDDPRAVNYIDSVPNSILVLALEPHLQSQFHPFIKITQAMYGGGLMSTRAQYLKSNGTVVFGRIIRDVDEHRTLNHVPVAYGVGSCAPKAVVKAVGTNVQLKILTSBGVTQTICPGDYIAGDNNGIVRIPVQETDISKLVTYIEKSIIEVDRLVSEAIKNGLPAKAAQTARRMVLKDYI	I32-19A: 208, 213, 218, 222, 225, 226, 229, 233
I32-19B SEQ ID NO:20	dimer	SGMRVYLGAHDHAGYELKQAIIFLKMGTGHEPIDCGALRYDADDDYPAFICIAAATRTVADPGSLGIVLGGSGNGEIQIAANKVPGARCALAWSVQTAALAREHNNAQLIGIGGRMHTLEALRIVKAFVTPWSKAQHRHRRIDILAEYERTHEAPPVPGAPA	I32-19B: 20, 23, 24, 27, 117, 118, 122, 125
I32-28A SEQ ID NO:21	trimer	GDDARIAAGIDVDELNSQIGVLLAEPLPDDVRAALSAIQHDLFDLGGELCIPGHAAITEDHLRLALWLHYNGQLPPLLEEFILPGGARGAALAHVCRVTCRRASERSIKALGASEPLNIAPAAAYNLLSDLLFVLARVLNRAAGGADVLDWDRTRAH	I32-28A: 60, 61, 64, 67, 68, 71, 110, 120, 123, 124, 128
I32-28B SEQ ID NO:22	dimer	ILSAEQSFTLRHPHGQAALAFVREPAALAGVQRLRLGLSDGEQVWGELLV RVPLLGEVDLPFRSEIVRTPQGAELRPLTLTGERAWVAVSGQATAAEGGEMAFAFQFQAHLATPEAEGEGGAFFVMVQAAAGVTLLLVAMALPQGLAAGLPAA	I32-28B: 35, 36, 54, 122

Name	Component Multimer	Amino Acid Sequence	Identified interface residues
			, 129, 137, 140, 141, 144, 148
I53-40A.1 SEQ ID NO:23	pentamer	TKKVGIVDTTTFARVDMASAAITLTKMESPNIKIIRKTVPGIKDLPVACKKLL EEEGCDIVMALGMPGKKEKDKVCAHEASLGLMLAQMLTNKHIIIEVFVHDEEA KDDAELKILAAARRAIEHALNVYLLFKPEYLTRMAGKGLRQGFEDAGPARE	I53-40A: 20, 23, 24, 27, 28, 109, 112, 1 13, 116, 120, 1 24
I53-40B.1 SEQ ID NO:24	trimer	DDINNQLKRLKVIPIVIAIDNAEDIIPLGKVLAEENGLPAAEITFRSSAAVKAI MLLRSAQPEMLIGAGTILNGVQALAAKEAGADFVVS PGFNPNTRACQIIIGI DIVPGVNNPSTVEQALEMGLTTLKFFPAEASGGISMVKSLVGPYGDIRLMPT GGITPDNIDNYLAIPQVLACGGTWMVDKLVNNGEWEIARLTREIVEQVNP	I53-40B: 47, 51, 54, 58, 74, 102
I53-47A.1 SEQ ID NO:25	trimer	PIFTLNTNIAKDDVPSDFLSLTSRLVGLILSKPGSYVAVHINTDQQLSFGGS TNPAAFGTLMISIGGIEPDKNRHSASVLFDFHLNAMLGIPKNRMYIHFNVLNGD DVGWNGTTF	I53-47A: 22, 25, 29, 72, 79, 86, 87
I53-47A.1NegT2 SEQ ID NO:26	trimer	PIFTLNTNIAKDDVPSDFLSLTSRLVGLILSEPGSYVAVHINTDQQLSFGGS TNPAAFGTLMISIGGIEPDKNRHSASVLFDFHLNAMLGIPKNRMYIHFNVLNGD DVGWNGTTF	I53-47A: 22, 25, 29, 72, 79, 86, 87
I53-47B.1 SEQ ID NO:27	pentamer	NQSHKDHETVRIAVVRARWHADIVDACVEAFEIAMAAIGGDRFAVDVFDVP GAYEIPHARTLAETGRYGAVLGTAFVVGNGGIYRHEFVASAVIDGMMNVQLD TGVVPLSAVLTTPHRYRDSDEHHRFFAAHFVAVKGVEAARACIEILNAREKIAA	I53-47B: 28, 31, 35, 36, 39, 131, 132, 1 35, 139, 146
I53-47B.1NegT2 SEQ ID NO:28	pentamer	NQSHKDHETVRIAVVRARWHADIVDACVEAFEIAMAAIGGDRFAVDVFDVP GAYEIPHARTLAETGRYGAVLGTAFVVDGGIYDHEFVASAVIDGMMNVQLD TGVVPLSAVLTTPHEYEDSDEHHEFFAAHFVAVKGVEAARACIEILNAREKIAA	I53-47B: 28, 31, 35, 36, 39, 131, 132, 1 35, 139, 146
I53-50A.1 SEQ ID NO:29	trimer	EELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLEITFTVPDADTVIKAL SVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFY MPGVMTPTELKAMKLGHDILKLPFGEVVGPPQFVKAMKGFPPNVKFPVPTGGV NLDNVCEWFKAGVLAVGVGDALVKGDPDEVREKAKKFVEKIRGCTE	I53-50A: 25, 29, 33, 54, 57
I53-50A.1NegT2 SEQ ID NO:30	trimer	EELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLEITFTVPDADTVIKAL SVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFY MPGVMTPTELKAMKLGHDILKLPFGEVVGPEFVEAMKGFPPNVKFPVPTGGV DLDVCEWFDAGVLAVGVGDALVKGDPDEVREDAKEFVEEIRGCTE	I53-50A: 25, 29, 33, 54, 57
I53-50A.1PostT1 SEQ ID NO:31	trimer	EELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLEITFTVPDADTVIKAL SVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFY MPGVMTPTELKAMKLGHDILKLPFGEVVGPPQFVKAMKGFPPNVKFPVPTGGV NLDNVCKWFKAGVLAVGVGKALVKGKDPDEVREKAKKFVKKIRGCTE	I53-50A: 25, 29, 33, 54, 57
I53-50B.1 SEQ ID NO:32	pentamer	NQSHKDHETVRIAVVRARWHAIEVDACVSFAFEAMRDIGGDRFAVDVFDVP GAYEIPHARTLAETGRYGAVLGTAFVVGNGGIYRHEFVASAVIDGMMNVQLD TGVVPLSAVLTTPHRYRDSADHTLLFLALFAVKGMEAAARACVEILAAREKIAA	I53-50B: 24, 28, 36, 124 , 125, 127, 128 , 129, 131, 132 , 133, 135, 139
I53-50B.1NegT2 SEQ ID NO:33	pentamer	NQSHKDHETVRIAVVRARWHAIEVDACVSFAFEAMRDIGGDRFAVDVFDVP GAYEIPHARTLAETGRYGAVLGTAFVVDGGIYDHEFVASAVIDGMMNVQLD TGVVPLSAVLTTPHEYEDSADHTLLFLALFAVKGMEAAARACVEILAAREKIAA	I53-50B: 24, 28, 36, 124 , 125, 127, 128 , 129, 131, 132 , 133, 135, 139
I53-50B.4PostT1 SEQ ID NO:34	trimer	NQSHKDHETVRIAVVRARWHAIEVDACVSFAFEAMRDIGGDRFAVDVFDVP GAYEIPHARTLAETGRYGAVLGTAFVVGNGGIYRHEFVASAVINGMMNVQLN TGVVPLSAVLTTPHNYDKSKAHTLLFLALFAVKGMEAAARACVEILAAREKIAA	I53-50B: 24, 28, 36, 124 , 125, 127, 128 , 129, 131, 132 , 133, 135, 139
I53-40A genus SEQ ID NO:35	pentamer	TKKVGIVDTTTFARVDMASAAITLTKMESPNIKIIRKTVPGIKDLPVACKKLL EEEGCDIVMALGMPGK (A/K) EKDKVCAHEASLGLMLAQMLTNKHIIIEVFH EDEAKDDAELKILAAARRAIEHALNVYLLFKPEYLTRMAGKGLRQGFEDAGP ARE	
I53-40B genus SEQ ID NO:36	trimer	(S/D) (T/D) INNQLK (A/R) LKVIPIVIAIDNAEDIIPLGKVLAEENGLPAAE ITFRSSAAVKAIMLLRSAQPEMLIGAGTILNGVQALAAKEAGA (T/D) FVVS PGFNPNTRACQIIIGIDIVPGVNNPSTVE (A/Q) ALEMGLTTLKFFPAEASG GISMVKSLVGPYGDIRLMPTGGITP (S/D) NIDNYLAIPQVLACGGTWMVDK KLV (T/R) NGEWEIARLTREIVEQVNP	
I53-47A genus SEQ ID NO:37	trimer	PIFTLNTNIKA (T/D) DVPSDFLSLTSRLVGLILS (K/E) PGSYVAVHINTD QQLSFGGSTNPAAFGTLMISIGGIEP (S/D) KN (R/E) DHSVLFDFHLNAMLG IPKNRMYIHFN (N/D) L (N/D) GDDVGVNGTTF	
I53-47B genus	pentamer	NQSHK (Y/H) ETVRIAVVRARWHADIVDACVEAFEIAMAAIGGDRFAVDV FDVPGAYEIPHARTLAETGRYGAVLGTAFV (N/D) GGIY (R/D) HEFVAS AVIDGMMNVQL (S/D) TGVVPLSAVLTTPH (R/E) Y (R/E) DS (A/D) E (H/D)	

Name	Component Multimer	Amino Acid Sequence	Identified interface residues
SEQ ID NO:38		)H(R/E)FFAAHFAVKGVEAARACIEIL(A/N)AREKIAA	
I53-50A genus SEQ ID NO:39	trimer	EELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIETFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGVMTPTTELKAMKLGH(T/D)ILKLPGEVVG(P/Q/E)FV(K/E)AMKGFPPNVKFVPTGGV(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	pentamer	NQHSKHD(Y/H)ETVRIAVVRARWHAETVDACVSAFEAAM(A/R)DIGGDRF AVDFVDVPGAYEIPLHARTLAETGRYGAVLGTAFFV(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)TLLFLALFAVKGMEAAACVETLAAREKIAA	
T32-28A SEQ ID NO:41	dimer	GEVPIGDPKELNGMEIAAVYLQPIEMEPRGIDLAASLADIHLEADHAKNNENGFFEGFWMPYLTIAYALANADTGAIKTGTLMMPVADDGPHYGANIAMEKD KKGFGVGTALYALTFLISNPEKQGGRHVDEETGVGKWFEPFVVITYFFKYTGTPK	
T32-28B SEQ ID NO:42	trimer	SQAIGILELTSIAKGMELGDAMLKSANVDLLVSKTISPGKFLMLGGDIGAIQQAIETGTSQAGEMLVDSLVLANIHPVSLPAISGLNSVDKQAVGIVETWSVAACISAADLAVKGSNVTIIVRHMAFGIGGKCYMVVAGDVLDAVAVATASLAGAKGLLVYASIIIPRHEAMWRQMVEG	
T33-09A SEQ ID NO:43	trimer	EEVVLITVPSALVAVKIAHALVEERLAACVNIVPGLTSIYRWQGSVVDHLEL LLLVKTTHAFPKLKERVKALHPYTVPEIVALPIAEGNREYLDWLRENTG	
T33-09B SEQ ID NO:44	trimer	VRGIRGAITVEEDTPAAILAATIELLLKMLEANGIQSYEELAAVIFTVTEDL TSAFPAAEARLIGMHRVPLLSAREVPVPGSLPRVIRVLALWNTDTPQDRVRH VYLNEAVRLRPDLESAQ	
T33-15A SEQ ID NO:45	trimer	SKAKIGIVTVSDRASAGITADISGKATILALNLYLTSEWEPIYQVIPDEQDV IETTLIKMADEQDCCLIVTTGGTGPAKRDVTPATEAVCDRMMPGFGELMRA ESLKEVPTAILSRQTAGLRGDSLIVNLPDGPASISDCLLAVFPAIYPICIDLM EGPYLECNEAMIKPFRPKAK	
T33-15B SEQ ID NO:46	trimer	VRGIRGAITVNSDTPSIIITATILLEKMLEANGIQSYEELAAVIFTVTEDL TSAFPAAEARLIGMHRVPLLSAREVPVPGSLPRVIRVLALWNTDTPQDRVRH VYLSEAVRLRPDLESAQ	
T33-21A SEQ ID NO:47	trimer	RITTKVGDGKSTRLFGGEEVWKDSPIIEANGTLDLTSFIGEAKHYVDEEMK GILEEIQNDIYKIMGEIGSKGKIEGISEERIAWLLKILRYMEMVNLKSFVL PGGTLESAKLDVCRTIARRALRKVLTVTREFGIGAEAAAYLLALSDLLFLLA RVIEIEKNKLKEVRS	
T33-21B SEQ ID NO:48	trimer	PHLVIETATNRLLETSPGELLEQANKALFASGQFGEADIKSRFVTLEAYRQG TAAVERAYLHACLSILDGRDIATRLLGASLCAVLAEAVAGGGEVGVSVSVE VREMERLSYAKRVVARQR	
T33-28A SEQ ID NO:49	trimer	ESVNTSFLSPSLVTIRDFDNGQFAVLIRIGRTGFPADKGDIDLCLDKMIGVRA AQIFLGDDTEDGFGKPHIRICVDIDDKHTYNAMVYVDLIVGTGASEVERET AEEEEAKLALRVALQVDIADEHSCVTQFEMKLEELSSDSFHPDKDEYYKDFL	
T33-28B SEQ ID NO:50	trimer	PVIQTFVSTPLDHHKRLLLAIYRIVTRVVLGKPEDLVMMTFHDSTPMHFFG STDVPACVRVEALGGYGPSEPEKVTISIVTAATAVCGIVADRIFVLYFSLH CGWNGTNF	
T33-31A SEQ ID NO:51	trimer	EEVVLITVPSALVAVKIAHALVEERLAACVNIVPGLTSIYREEGSVVDHLEL LLLVKTTHDAFPKLERVKELHPYEVPEIVALPIAEGNREYLDWLRENTG	
I53-50A ΔCys SEQ ID NO: 53	trimer	EELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIETFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVMTPTTELKAMKLGHITILKLPGEVVGPFVKAMKGFPPNVKFVPTGGV NLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE	
T33_dn2A SEQ ID NO: 135		NLAEMYKAGNAMYRKQYTTIAITAYTLALLKDPNNAAEAWYNLGNAYYKQGD YDEAIEAYQKALEDPNNAAEAWYNLGNAYYKQGDYDEAIEYYKKALRLDPRN YDAIENLIEAEKQG	
T33_dn2B SEQ ID NO: 136		EEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAAEAWYNLGNAYYKQGD YREAIRYYLRALKLDPENAAEAWYNLGNALYKQGGYDLATIAAYQAALDEDPNN AEAKQNLGNKQKQ	
T33_dn5A SEQ ID NO: 137		NSAEAMYKMGNAAYKQGDYILAIYALLALEKDPNNAAEAWYNLGNAYYKQGD YDEAIEYYQKALEDPNNAAEAWYNLGNAYYKQGDYDEAIEYYEKALELDPNN AEALKNLLEAIAEQD	
T33_dn5A SEQ ID NO: 138		TDPLAVITLYTIALKAEKSIARAKAAEALGKIGDERAVEPLIKALKDEEDALVR AAAADALGQIGDERAVEPLIKALKDEEGLVRSAAIALGQIGDERAVQPLIK ALTDERDLVRVAAVALGRIGDEKAVRPLIIVLKDEEGEVREAAAIALGSIG GERVRAAMEKLAERGTFGARKVAVNYLETHK	
T33_dn10A SEQ ID NO: 139		EEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAAEAWYNLGNAYYKQGD YDEAIEYYQKALELDPNNAAEAWYNLGNAYYKQGDYDEAIEYYEKALELDPEN LEALQNLNAMDKQ	

Name	Component Multimer	Amino Acid Sequence	Identified interface residues
T33_dn10B SEQ ID NO: 140		IEEVVAEMIDILAESSKKSIIEELARAADNKTEKAVAEIEEIALATAAIQ LIEALAKNLASEEFMARAI SAIAELAKKAIEAIYRLADNHTTDTFMARIAA IANLAVTAI LAIAALASNHTTEEFMARAI SAIAELAKKAIEAIYRLADNHTT DKFMAAAIEAIALLATLAILAIALASNHTTEKFMAAIMAIAILAAKAIEA IYRLADNHTSPYIEKAIEAIEKIARKAKAIEMLAKNITTEYKEKAKKII DIIRKLAKMAIKKLEDNRT	
I53_dn5A SEQ ID NO: 141		KYDGSKLRI GILHARWNAEII LALVLGALKRLQEFVVKRENIIE TVPGSFE LPYGSKL FVEKQKRLGKPLDAIIPIGVLIKSTMHFEYICDSTTHQLMKLNF ELGIPVIFGVLTCLTDEQAEARAGLIEGKMHNHGEDWGAAAVEMATKFN	
I53_dn5B SEQ ID NO: 142		EEAELAYLLGELAYKLGEYRIATRAYRIALKRDPNNAEAWYNLGNAYYKQGR YREAIEYYQKALELDPNNAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNN ADAMQNLNNAKMREE	

[0111] Table 1 provides the amino acid sequence of the first polypeptide and second polypeptides of embodiments of the present disclosure. In each case, the pairs of sequences together form an I53 multimer with icosahedral symmetry. The right hand column in Table 1 identifies the residue numbers in each illustrative polypeptide that were identified as present at the interface of resulting assembled virus-like particles (*i.e.*: “identified interface residues”). As can be seen, the number of interface residues for the illustrative polypeptides of SEQ ID NO:1-34 range from 4-13. In various embodiments, the first polypeptide 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. SEQ ID NOS: 35-51 represent other amino acid sequences of the first polypeptide and second polypeptides from embodiments of the present disclosure. In other embodiments, the first polypeptide and/or 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.

[0112] 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 virus-like particles: 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, 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.

[0113] In various embodiments of the VLPs of the invention, the first polypeptide and second polypeptides, or the vice versa, 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%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100% over its length, and/or identical at least at one identified interface position, to the amino acid sequence indicated by the SEQ ID NO):

SEQ ID NO:1 and SEQ ID NO:2 (I53-34A and I53-34B);
 SEQ ID NO:3 and SEQ ID NO:4 (I53-40A and I53-40B);
 SEQ ID NO:3 and SEQ ID NO:24 (I53-40A and I53-40B.1);
 SEQ ID NO:23 and SEQ ID NO:4 (I53-40A.1 and I53-40B);
 SEQ ID NO:35 and SEQ ID NO:36 (I53-40A genus and I53-40B genus);
 SEQ ID NO:5 and SEQ ID NO:6 (I53-47A and I53-47B);
 SEQ ID NO:5 and SEQ ID NO:27 (I53-47A and I53-47B.1);
 SEQ ID NO:5 and SEQ ID NO:28 (I53-47A and I53-47B.1NegT2);
 SEQ ID NO:25 and SEQ ID NO:6 (I53-47A.1 and I53-47B);
 SEQ ID NO:25 and SEQ ID NO:27 (I53-47A.1 and I53-47B.1);
 SEQ ID NO:25 and SEQ ID NO:28 (I53-47A.1 and I53-47B.1NegT2);
 SEQ ID NO:26 and SEQ ID NO:6 (I53-47A.1NegT2 and I53-47B);
 SEQ ID NO:26 and SEQ ID NO:27 (I53-47A.1NegT2 and I53-47B.1);
 SEQ ID NO:26 and SEQ ID NO:28 (I53-47A.1NegT2 and I53-47B.1NegT2);
 SEQ ID NO:37 and SEQ ID NO:38 (I53-47A genus and I53-47B genus);
 SEQ ID NO:7 and SEQ ID NO:8 (I53-50A and I53-50B);
 SEQ ID NO:7 and SEQ ID NO:32 (I53-50A and I53-50B.1);
 SEQ ID NO:7 and SEQ ID NO:33 (I53-50A and I53-50B.1NegT2);
 SEQ ID NO:7 and SEQ ID NO:34 (I53-50A and I53-50B.4PosT1);
 SEQ ID NO:29 and SEQ ID NO:8 (I53-50A.1 and I53-50B);

SEQ ID NO:29 and SEQ ID NO:32 (I53-50A.1 and I53-50B.1);
 SEQ ID NO:29 and SEQ ID NO:33 (I53-50A.1 and I53-50B.1NegT2);
 SEQ ID NO:29 and SEQ ID NO:34 (I53-50A.1 and I53-50B.4PosT1);
 SEQ ID NO:30 and SEQ ID NO:8 (I53-50A.1NegT2 and I53-50B);
 SEQ ID NO:30 and SEQ ID NO:32 (I53-50A.1NegT2 and I53-50B.1);
 SEQ ID NO:30 and SEQ ID NO:33 (I53-50A.1NegT2 and I53-50B.1NegT2);
 SEQ ID NO:30 and SEQ ID NO:34 (I53-50A.1NegT2 and I53-50B.4PosT1);
 SEQ ID NO:31 and SEQ ID NO:8 (I53-50A.1PosT1 and I53-50B);
 SEQ ID NO:31 and SEQ ID NO:32 (I53-50A.1PosT1 and I53-50B.1);
 SEQ ID NO:31 and SEQ ID NO:33 (I53-50A.1PosT1 and I53-50B.1NegT2);
 SEQ ID NO:31 and SEQ ID NO:34 (I53-50A.1PosT1 and I53-50B.4PosT1);
 SEQ ID NO:39 and SEQ ID NO:40 (I53-50A genus and I53-50B genus);
 SEQ ID NO:9 and SEQ ID NO:10 (I53-51A and I53-51B);
 SEQ ID NO:11 and SEQ ID NO:12 (I52-03A and I52-03B);
 SEQ ID NO:13 and SEQ ID NO:14 (I52-32A and I52-32B);
 SEQ ID NO:15 and SEQ ID NO:16 (I52-33A and I52-33B);
 SEQ ID NO:17 and SEQ ID NO:18 (I32-06A and I32-06B);
 SEQ ID NO:19 and SEQ ID NO:20 (I32-19A and I32-19B);
 SEQ ID NO:21 and SEQ ID NO:22 (I32-28A and I32-28B);
 SEQ ID NO:23 and SEQ ID NO:24 (I53-40A.1 and I53-40B.1);
 SEQ ID NO:41 and SEQ ID NO:42 (T32-28A and T32-28B);
 SEQ ID NO:43 and SEQ ID NO:44 (T33-09A and T33-09B);
 SEQ ID NO:45 and SEQ ID NO:46 (T33-15A and T33-15B);
 SEQ ID NO:47 and SEQ ID NO:48 (T33-21A and T33-21B);
 SEQ ID NO:49 and SEQ ID NO:50 (T33-28A and T32-28B); and
 SEQ ID NO:51 and SEQ ID NO:44 (T33-31A and T33-09B (also referred to as T33-31B))

[0114] In some embodiments, the one or more hMPV F proteins, or antigenic fragments thereof, are expressed as a fusion protein with the first multimerization domain. In some embodiments, the first multimerization domain and the hMPV F protein ectodomain are joined by a linker sequence. In some embodiments, the linker sequence comprises a foldon, wherein the foldon sequence is EKAAKAEEAARK (SEQ ID NO: 125).

[0115] Non-limiting examples of designed protein complexes useful in protein-based VLPs of the present disclosure include those disclosed in U.S. Patent No. 9,630,994; Int'l Pat. Pub. No. WO2018187325A1; U.S. Pat. Pub. No. 2018/0137234 A1; U.S. Pat. Pub. No. 2019/0155988 A2, each of which is incorporated herein in its entirety.

[0116] In various embodiments of the VLPs of the disclosure, the multimerization domains are 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%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, at least 100% over its length, and/or identical at least at one identified interface position, to the amino acid sequence indicated by the SEQ ID NO):

SEQ ID NO: 135 and SEQ ID NO: 136 (T33_dn2A and T33_dn2B);

SEQ ID NO: 137 and SEQ ID NO: 138 (T33_dn5A and T33_dn5B);

SEQ ID NO: 139 and SEQ ID NO: 140 (T33_dn10A and T33_dn10B); or

SEQ ID NO: 141 and SEQ ID NO: 142 (I53_dn5A and I53_dn5B).

Antigenic Proteins

[0117] The present disclosure provides protein-based VLP vaccines for hMPV in humans or animals (in particular pets, agricultural animals, and any animal linked to spread of the disease). The present disclosure relates to incorporation of any of antigenic fragment of hMPV F protein—*e.g.*, the ectodomain or an antigenic fragment thereof—into VLP vaccines. Guidance is particularly available from studies of the immune response to infection or vaccination, such as isolation of binding or neutralizing antibodies, genetic analysis of F protein sequence, structural studies of antigenic proteins and antibodies, and most particularly clinical and veterinary experience with subunit vaccines. Subunit vaccine for hMPV can be adapted for use with the VLPs of the present disclosure by employing the display modalities provided above. The disclosure refers to the ectodomain of hMPV F but it will be understood that portions of the transmembrane domain may be included.

[0118] The term “antigenic fragment” refers to any fragment of a protein that generates an immune response (humoral or T cell response) to the protein *in vivo*. The antigenic fragment may be a linear epitope, discontinuous epitope, or a conformation epitope (*e.g.*, a folded domain). The antigenic fragment may preserve the secondary, tertiary, and/or quaternary structure of the full-length protein. In some embodiments, the antigenic fragment comprises a

neutralizing epitope. In such cases, the VLP may generate a neutralizing antibody response. Antigenic fragments may be designed computationally, such as by predicting the secondary structure and rationally removing N- or C-terminal unstructured regions or internally loops, or entire structural elements (alpha helices and/or beta sheets). **FIG. 2** provides an illustrative secondary structure map. In some embodiments, the ectodomain comprises a truncation or deletion of some or all of the N-terminal signal peptide (*e.g.*, MSWKVVIIFSLLITPQHGL (SEQ ID NO: 133) or MSWKVMIISLLITPQHGL (SEQ ID NO: 134).

[0119] In some embodiments, the hMPV F protein ectodomain is a C-terminal truncation. In some embodiments, the hMPV F protein ectodomain sequence terminates at residue 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, or 490 of the native sequence.

[0120] The native sequence of the A strain hMPV F protein (GenBank AY145297) is shown below with the signal sequence underlined and italicized and the transmembrane and intracellular portion underlined (SEQ ID NO: 56):

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1  MSWKVVIIFS LLITPQHGLK ESYLEESCST ITEGYLSVLR
41 TGWYTNVFTL EVGDVENLTC SDGPSLIKTE LDLTKSALRE
81 LKTVSADQLA REEQIENPRQ SRFVLGAIAL GVATAAAVTA
121 GVAIAKTIRL ESEVTAIKNA LKTTNEAVST LGNGVRVLAT
161 AVRELKDFVS KNLTRAINKN KCDIDDLKMA VSFSQFNRRF
201 LNVVRQFSDN AGITPAISLD LMTDAELARA VSNMPTSAGQ
241 IKLMLENRAM VRRKGFGILI GVGSSVIYM VQLPIFGVID
281 TPCWIVKAAP SCSGKKGNYA CLLREDQGWY CQNAGSTVYY
321 PNEKDCETRG DHVFCDTAAG INVAEQSKEC NINISTTNYP
361 CKVSTGRHPI SMVALSPLGA LVACYKGVSC SIGSNRVGII
401 KQLNKGCSYI TNQDADTVTI DNTVYQLSKV EGEQHVIGR
441 PVSSSFDPK FPEQDFNVAL DQVFENIENS QALVDQSNRI
481 LSSAEKGNTG FIIVIIILIAV LGSSMILVSI FIIIKKTKPK
521 TGAPPELSGV TNNGFIPHS (SEQ ID NO: 56)

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[0121] The native signal sequence is post-translationally cleaved when the protein is expressed. The native signal sequence may be replaced with another signal sequence for expression of the ectodomain, or in some embodiments no signal sequence is used. Thus, in some embodiments, the hMPV F protein ectodomain is SEQ ID NO: 57, or a variant thereof:

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1                                     K ESYLEESCST ITEGYLSVLR
41 TGWYTNVFTL EVGDVENLTC SDGPSLIKTE LDLTKSALRE
81 LKTVSADQLA REEQIENPRQ SRFVLGAIAL GVATAAAVTA
121 GVAIAKTIRL ESEVTAIKNA LKTTNEAVST LGNGVRVLAT
161 AVRELKDFVS KNLTRAINKN KCDIDDLKMA VSFSQFNRRF
201 LNVVRQFSDN AGITPAISLD LMTDAELARA VSNMPTSAGQ
241 IKLMLENRAM VRRKGFGILI GVGSSVIYM VQLPIFGVID
281 TPCWIVKAAP SCSGKKGNYA CLLREDQGWY CQNAGSTVYY

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321 PNEKDCETRG DHVFCDTAAG INVAEQSKEC NINISTTNYP
361 CKVSTGRHPI SMVALSPLGA LVACYKGVSC SIGSNRVGII
401 KQLNKGCSYI TNQDADTVTI DNTVYQLSKV EGEQHVIGR
441 PVSSSFDPK FPEQDFNVAL DQVFENIENS QALVDQSNRI
481 LSSAEKGNTG F
521

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(SEQ ID NO: 57)

[0122] In some embodiments, the hMPV F protein ectodomain shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 57, or an antigenic fragment thereof.

[0123] Further hMPV F protein sequences are provided in Table 2. In some embodiments, the hMPV F protein ectodomain shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an ectodomain in Table 2, or an antigenic fragment thereof.

[0124] Various signal sequences can be used, paired with the native ectodomain sequence or swapped between different hMPV sequences. Thus, in some embodiments, the antigen comprises a sequence that shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to a signal sequence in Table 2.

[0125] The hMPV F protein may comprise one or more substitutions.

[0126] Illustrative substitutions include A185P, Q100R, S101R, T127C, N153C, V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, G106 deletion, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, I177L, K450A, S470A, G294E, T365C, V463C, L219K, H368N, , and/or V231I relative to SEQ ID NO: 57. Thus, in various embodiments, the hMPV F protein ectodomain comprises one or more substitutions selected from this list.

[0127] Illustrative substitutions include V84C, A140C, A147C, N97G, P98G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, R99G, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, Q100R, S101R, G106 deletion, S101R, A185P, I177L, and G294E relative to SEQ ID NO: 57. Thus, in various embodiments, the hMPV F protein ectodomain comprises one or more substitutions selected from this list.

[0128] Illustrative substitutions include V84C, A140C, A147C, N97G, P98G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, R99G, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, Q100R, S101R, G106 deletion, S101R, A185P, I177L, and G294E. Thus, in various embodiments, the hMPV F protein ectodomain comprises two or more substitutions selected from this list.

[0129] Illustrative substitutions include A185P, Q100R, S101R, T127C, N153C, T365C, V463C, L219K, V231I, G294E, N153C, N97G, P98G, R99G, Q100G, S101G, and R102G.

Thus, in various embodiments, the hMPV F protein ectodomain comprises one or more substitutions selected from this list.

[0130] Illustrative substitutions include A185P, Q100R, S101R, T127C, N153C, T365C, V463C, L219K, V231I, G294E, N153C, N97G, P98G, R99G, Q100G, S101G, and R102G. Thus, in various embodiments, the hMPV F protein ectodomain comprises two or more substitutions selected from this list.

[0131] In some embodiments, the hMPV F protein ectodomain comprises the substitutions Q100R and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A185P, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A185P, T127C, N153C, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, and R102G. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, and R102G. In some embodiments, the hMPV F protein ectodomain comprises the substitutions and deletion A63C, A140C, A147C, K188C, G106 deletion, N97G, P98G, R99G, Q100G, S101G, and R102G. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A113C, A120C, A339C, Q426C, T160F, I177L, Q100K, and S101A. In some embodiments, the hMPV F protein ectodomain comprises the substitutions V84C, A140C, A147C, A249C, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A63C, A140C, A147C, K188C, K450C, S470C, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions and deletion A63C, A140C, A147C, K188C, G106 deletion, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A113C, A120C, A339C, Q426C, T160F, I177L, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A185P, A113C, A339C, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A185P, T160F, I177L, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A185P, A113C, A339C, T160F, I177L, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A63C, K188C, N97G, P98G, R99G, Q100G, S101G, and R102G. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A63C, K188C, K450A, S470A, N97G, P98G, R99G, Q100G, S101G, and R102G. In some embodiments, the hMPV F protein ectodomain comprises the substitutions

A63C, A140C, A147C, K188C, G294E, N97G, P98G, R99G, Q100G, S101G, and R102G. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G, and G294E. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A63C, K188C, N97G, P98G, R99G, Q100G, S101G, R102G, and G294E. In some embodiments, the hMPV F protein ectodomain comprises the substitutions T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, Q100R, and S101R. In some embodiments, the hMPV F protein ectodomain comprises the substitutions A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, and R102G. In some embodiments, the hMPV F protein ectodomain comprises the substitutions V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, and R102G. In some embodiments, the hMPV F protein ectodomain comprises the substitutions T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, N97G, P98G, R99G, Q100G, S101G, H368N, and R102G.

[0132] Further illustrative amino acid substitutions are provided in Error! Reference source not found.. In various embodiments, the ectodomain comprises one or more substitutions selected from substitutions and alternative substitutions listed in Error! Reference source not found.. In some embodiments, the substitutions has the intended effect on the VLP, it being understood that the intended effect is not achieved in every embodiment of the VLP having that substitution. In some embodiments, the intended effect is increased expression in a production cell line (Mas et al. PLoS Pathog. 12: e1005859 (2016)). In some embodiments, the intended effect is to stabilize a post-fusion or pre-fusion form of the hMPV F protein ectodomain (Battles et al. Nat Commun. 8:1528 (2017)). In some embodiments, the intended effect is binding neutralizing antibodies against hMPV F protein. The antigen may further comprise a Leucine at the N-terminus, as set forth in SEQ ID NOs: 146-178.

Table 2. Illustrative hMPV Protein F Ectodomain Antigenic Variants

SEQ ID NO:	Ectodomain	hMPV Antigen Mutations	C-term F protein Termination Residue
58	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD GPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAV STLGNVRLVLAATVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMQLPFIG VIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCNAGSTVYY PNEKDCETRGDHFCDTAAGINVAEQSKECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQALVDQSNRIL	A185P, Q100R, S101R	481

SEQ ID NO:	Ectodomain	hMPV Antigen Mutations	C-term F protein Termination Residue
59	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD GPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAV STLGNVRLVATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMQLPPIFG VIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQNA GSTVYY PNEKDCETR GDHVFCDTAAGINVAEQSKECNINISTTNYPCVK STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	A185P, Q100R, S101R	472
60	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD GPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAV STLGNVRLVATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMQLPPIFG VIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQNA GSTVYY PNEKDCETR GDHVFCDTAAGINVAEQSKECNINISTTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	A185P, Q100R, S101R	489
61	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD GPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAV STLGNVRLVATAVRELKDFVSKNLTRAINKNKCDIADLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMQLPPIFG VIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQNA GSTVYY PNEKDCETR GDHVFCDTAAGINVAEQSKECNINISTTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	Q100R, S101R	472
62	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD GPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAKCI RLESEVTAIKNALKKTNEAV STLGCGRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMQLPPIFG VIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQNA GSTVYY PNEKDCETR GDHVFCDTAAGINVAEQSKECNINISTTNYPCKV STGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	A185P, T127C, N153C, Q100R, S101R	472
63	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD GPSLIKTELDTKSALRELKTCADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTNECV STLGNVRLVATAVRELKEFVSKNLTSAINKNKCDIADLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRCMVRRKGFGILIGVYGSSVIYMQLPPIFG VIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYY PNDKDCETR GDHVFCDTAAGINVAEQSRECNINISTTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G	472

SEQ ID NO:	Ectodomain	hMPV Antigen Mutations	C-term F protein Termination Residue
64	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD GPSLIKTELDTKSALRELKTCADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTTNECV STLGNGVRVLATAVRELKEFVSKNLTSAINKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRCMVRRKGFGILIGVYGSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKDGNACLLREDQGWYCKNAGSTVYY PNDKDCETRGDHFVCDTAAGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRPVSSSFDPI KFPEDQFNVALDQVFESIENSQALVDQSNKILNSAESAI	V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G	488
65	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD CPSLIKTELDTKSALRELKTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTTNECV STLGNGVRVLATAVRELKEFVSKNLTSAINKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKDGNACLLREDQGWYCKNAGSTVYY PNDKDCETRGDHFVCDTAAGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRPVSSSFDPI CFPEDQFNVALDQVFESIENCQA	A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G	472
66	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD CPSLIKTELDTKSALRELKTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTTNECV STLGNGVRVLATAVRELKEFVSKNLTSAINKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKDGNACLLREDQGWYCKNAGSTVYY PNDKDCETRGDHFVCDTAAGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRPVSSSFDPI CFPEDQFNVALDQVFESIENCQALVDQSNKILNSAESAI	A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G	488
67	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCS CPSLIKTELDTKSALRELKTVSADQLAREEQIEGGGGGGFVL AIALGVATAAAVTAGVAIAKTIRLESEVTAIKNCLKTTNECVS TLGNGVRVLATAVRELKDFVSKNLTSAINKNKCDIDDLMAVS FSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMP TSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMQLPFIGV IDTPCWIIKAAPSCSGKKGNACLLREDQGWYCKNAGSTVYY NEKDCETRGDHFVCDTAAGINVAEQSKECNINISTNYPCKVS TGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIKQLNKG SYITNQDADTVTIDNTVYQLSKVEGEQHVIGRPVSSSFDPIK FPEDQFNVALDQVFENIENSQA	A63C, A140C, A147C, K188C, G106 deleted, N97G, P98G, R99G, Q100G, S101G, R102G	472
68	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCS CPSLIKTELDTKSALRELKTVSADQLAREEQIEGGGGGGFVL AIALGVATAAAVTAGVAIAKTIRLESEVTAIKNCLKTTNECVS TLGNGVRVLATAVRELKDFVSKNLTSAINKNKCDIDDLMAVS FSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMP TSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMQLPFIGV	A63C, A140C, A147C, K188C, G106	488

SEQ ID NO:	Ectodomain	hMPV Antigen Mutations	C-term F protein Termination Residue
	IDTPCWIIKAAPSCSGKKGNACLLREDQGWYCNAGSTVYYP NEKDCETRGDHFVCDTAAGINVAEQSKECNINISTNYPCKVS TGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP KFPEDQFNVALDQVFENIENSQALVDQSNRILSSAESAI	deleted, N97G, P98G, R99G, Q100G, S101G, R102G	
69	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD GPSLIKTELDTKSALRELKTVSADQLAREEQIENPRKARFVL GAIALGVCTAAAVTCGIAIAKTIRLESEVNNAIKGALKTTNEAV STLGNGVRVLAFVAVRELKEFVSKNLTSAINKNKCDIADLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKMLLENRAMVRRKGFGILIGVYGSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYP PNDKDCETRGDHFVCDTACGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKG CSYITNQDADTVTIDNTVYCLSKVEGEQHVIKGRPVSSSFDP KFPEDQFNVALDQVFESIENSQA	A113C, A120C, A339C, Q426C, T160F, I17L, Q100K, S101A	472
70	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD GPSLIKTELDTKSALRELKTVSADQLAREEQIENPRKARFVL GAIALGVCTAAAVTCGIAIAKTIRLESEVNNAIKGALKTTNEAV STLGNGVRVLAFVAVRELKEFVSKNLTSAINKNKCDIADLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKMLLENRAMVRRKGFGILIGVYGSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYP PNDKDCETRGDHFVCDTACGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKG CSYITNQDADTVTIDNTVYCLSKVEGEQHVIKGRPVSSSFDP KFPEDQFNVALDQVFESIENSQA	A113C, A120C, A339C, Q426C, T160F, I177L, Q100K, S101A	488
71	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGW YTNVFTLEVGDVENLTCTDGPSLIKTELDTKSALRELKTC DQLAREEQIENPRRRFVLGAIALGVATAAAVTAAGIAIAKTIR LESEVNNAIKGCLKTTNECVSTLGNGVRVLATAVRELKEFVSKN LTSAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITP AISLDLMTDAELARAVSYMPTSAGQIKMLLENRCMVRRKGFGI LIGVYGSSVIYMQLPFIGVIDTPCWIIKAAPSCSEKDGNYAC LLREDQGWYCKNAGSTVYYPNDKDCETRGDHFVCDTAAGINVA EQSRECNINISTNYPCKVSTGRHPISMVALSPLGALVACYKG VSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKV EGEQHVIKGRPVSSSFDPKFPEDQFNVALDQVFESIENSQA	V84C, A140C, A147C, A249C, Q100R, S101R	472
72	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD CPSLIKTELDTKSALRELKTVSADQLAREEQIENPRRRFVL GAIALGVATAAAVTAAGIAIAKTIRLESEVNNAIKGCLKTTNECV STLGNGVRVLATAVRELKEFVSKNLTSAINKNKCDIADLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKMLLENRAMVRRKGFGILIGVYGSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYP PNDKDCETRGDHFVCDTAAGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP CFPEDQFNVALDQVFESIENCQA	A63C, A140C, A147C, K188C, K450C, S470C, Q100R, S101R	472
73	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCS CPSLIKTELDTKSALRELKTVSADQLAREEQIENPRRRFVL AIALGVATAAAVTAAGIAIAKTIRLESEVTAIKNCLKTTNECVS TLGNGVRVLATAVRELKDFVSKNLTSAINKNKCDIDDLMAVS FSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNMP	A63C, A140C, A147C, K188C,	472

SEQ ID NO:	Ectodomain	hMPV Antigen Mutations	C-term F protein Termination Residue
	TSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMQLPPIFGV IDTPCWIIKAAPSCSGKKGNACLLREDQGWYCNAGSTVYYP NEKDCETRGRDHVFCDTAAGINVAEQSKECNINISTNYPCKVS TGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG SYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPK KFPEDQFNVALDQVFENIENSQA	G106 deleted, Q100R, S101R	
74	KESYLEESCSTITEGYLSVLRGTWYTNVFTLEVGDVENLTCTD GPSLIKTELDLTSAKRELKTVSADQLAREEQIENPRRRRFVL GAIALGVCTAAAVTCGIAIAKTIRLESEVNAIKALKTTNEAV STLGNVVRVLAFAVRELKEFVSKNLTSALNKNKCDIADLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMQLPPIFG VIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYY PNDKDCETRGRDHVFCDTACGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKG CSYITNQDADTVTIDNTVYCLSKVEGEQHVIKGRPVSSSFDPK KFPEDQFNVALDQVFESIENSQA	A113C, A120C, A339C, Q426C, T160F, I177L, Q100R, S101R	472
75	KESYLEESCSTITEGYLSVLRGTWYTNVFTLEVGDVENLTCTD GPSLIKTELDLTSAKRELRTVSADQLAREEQIENPRRRRFVL GAIALGVCTAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAV STLGNVVRVLAFAVRELKDFVSKNLTRALNKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMQLPPIFG VIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCNAGSTVYY PNEKDCETRGRDHVFCDTACGINVAEQSKECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	A185P, A113C, A339C, Q100R, S101R	472
76	KESYLEESCSTITEGYLSVLRGTWYTNVFTLEVGDVENLTCTD GPSLIKTELDLTSAKRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAV STLGNVVRVLAFAVRELKDFVSKNLTRALNKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMQLPPIFG VIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCNAGSTVYY PNEKDCETRGRDHVFCDTAAGINVAEQSKECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	A185P, T160F, I177L, Q100R, S101R	472
77	KESYLEESCSTITEGYLSVLRGTWYTNVFTLEVGDVENLTCTD GPSLIKTELDLTSAKRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAV STLGNVVRVLAFAVRELKDFVSKNLTRALNKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYGSSVIYMQLPPIFG VIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCNAGSTVYY PNEKDCETRGRDHVFCDTACGINVAEQSKECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	A185P, A113C, A339C, T160F, I177L, Q100R, S101R	472
78	KESYLEESCSTITEGYLSVLRGTWYTNVFTLEVGDVENLTCTD CPSLIKTELDLTSAKRELKTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGIAIAKTIRLESEVNAIKALKTTNEAV STLGNVVRVLAFAVRELKEFVSKNLTSALNKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRAMVRRKGFGILIGVYGSSVIYMQLPPIFG VIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYY	A63C, K188C, N97G, P98G, R99G, Q100G,	472

SEQ ID NO:	Ectodomain	hMPV Antigen Mutations	C-term F protein Termination Residue
	PNDKDCETRGDHFVCDTAAGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRVPVSSSFDPI KFPEDQFNVALDQVFESIENSAQA	S101G, R102G	
79	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD CPSLIKTELDTKSALRELKTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAAGIAIAKTIRLESEVNAIKGALKTTNEAV STLGNGVRVLATAVRELKEFVSKNLTSAINKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRAMVRRKGFGILIGVYSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYY PNDKDCETRGDHFVCDTAAGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRVPVSSSFDPI AFPEDQFNVALDQVFESIENSAQA	A63C, K188C, K450A, S470A, N97G, P98G, R99G, Q100G, S101G, R102G	472
80	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCS CPSLIKTELDTKSALRELKTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAAGIAIAKTIRLESEVTAIKNCLKTTNECV STLGNGVRVLATAVRELKDFVSKNLTSAINKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGILIGVYSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKKGNYACLLREDQGWYCKNAGSTVYY PNEKDCETRGDHFVCDTAAGINVAEQSKECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRVPVSSSFDPI KFPEDQFNVALDQVFENIENSAQA	A63C, A140C, A147C, K188C, G294E, N97G, P98G, R99G, Q100G, S101G, R102G	472
81	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD CPSLIKTELDTKSALRELRTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAAGIAIAKTIRLESEVTAIKNCLKTTNECV STLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYSSVIYMQLPFIG VIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCKNAGSTVYY PNEKDCETRGDHFVCDTAAGINVAEQSKECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRVPVSSSFDPV CFPEDQFNVALDQVFESIENCAQA	A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G, G294E	472
82	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD CPSLIKTELDTKSALRELRTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAAGIAIAKTIRLESEVTAIKNALKTTNEAV STLGNGVRVLATAVRELKDFVSKNLTRAINKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSNM PTSAGQIKLMLENRAMVRRKGFGFLIGVYSSVIYMQLPFIG VIDTPCWIVKAAPSCSEKKGNYACLLREDQGWYCKNAGSTVYY PNEKDCETRGDHFVCDTAAGINVAEQSKECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIGRVPVSSSFDPV KFPEDQFNVALDQVFESIENSAQA	A63C, K188C, N97G, P98G, R99G, Q100G, S101G, R102G, G294E	472
83	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCAD GPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAAGIAIAKTIRLESEVTAIKNALKTTNEAV STLGCGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV	T127C, N153C, T365C, V463C,	472

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	SFSQFNRRFLNVVRQFSDNAGITPAISKDLMTDAELARAI SNM PTSAGQIKLMLENRAMVRRKGFGLIGVYGSSVIYMQLP IFG VIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCQNA GSTVYY PNEKDCETR GDHVFCDTAAGINVAEQSKECNINISTNYPCKV SCGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQCFESIENSQA	A185P, L219K, V231I, G294E, H368N, Q100R, S101R	
84	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVG DVENLTCAD GPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAK CIRLESEVTAIKNALKKTNEAV STLGCGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISKDLMTDAELARAI SNM PTSAGQIKLMLENRAMVRRKGFGLIGVYGSSVIYMQLP IFG VIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCQNA GSTVYY PNEKDCETR GDHVFCDTAAGINVAEQSKECNINISTNYPCKV SCGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQCFESIENSQAALVDQSNRILSSAEKGNTG	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, Q100R, S101R	490
85	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVG DVENLTCTD CPSLIKTELDLTKSALRELKTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGVAIAK TIRLESEVNAIKGCLKTTNECV STLGNGVRVLATAVRELKEFVSKNLTSAINKNKCDIADLCMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRAMVRRKGFGLIGVYGSSVIYMQLP IFG VIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYY PNDKDCETR GDHVFCDTAAGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDP I CFPEDQFNVALDQVFESIENCQA	A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G	472
86	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVG DVENLTCAD GPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAK CIRLESEVTAIKNALKKTNEAV STLGCGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISKDLMTDAELARAI SNM PTSAGQIKLMLENRAMVRRKGFGLIGVYGSSVIYMQLP IFG VIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCQNA GSTVYY PNEKDCETR GDHVFCDTAAGINVAEQSKECNINISTNYPCKV SCGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQCFESIENSQA	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, Q100R, S101R	472
87	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVG DVENLTCAD GPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRRRRFVL GAIALGVATAAAVTAGVAIAK CIRLESEVTAIKNALKKTNEAV STLGCGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISKDLMTDAELARAI SNM PTSAGQIKLMLENRAMVRRKGFGLIGVYGSSVIYMQLP IFG VIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCQNA GSTVYY PNEKDCETR GDHVFCDTAAGINVAEQSKECNINISTNYPCKV SCGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIIKQLNKG	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E,	490

SEQ ID NO:	Ectodomain	hMPV Antigen Mutations	C-term F protein Termination Residue
	CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQCFESIENSQALVDQSNRILSSAEKGNTG	Q100R, S101R	
88	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCTD GPSLIKTELDTKSALRELKTCADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGIAIAKTIKRLSEVNAIKGLKTTNECV STLGNGVRVLATAVRELKEFVSKNLTSAINKNKCDIADLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISLDLMTDAELARAVSYM PTSAGQIKLMLENRCMVRRKGFGLIGVYGSSVIYMQLPFIG VIDTPCWIIKAAPSCSEKDGNACLLREDQGWYCKNAGSTVYY PNDKDCETRGDHFVCDTAAGINVAEQSRECNINISTNYPCKV STGRHPISMVALSPLGALVACYKGVSCSIGSNRVGIKQLPKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQVFESIENSQA	V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G	472
89	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCD GPSLIKTELDTKSALRELRTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGVAIAKCIKRLSEVTAIKNALKKTNEAV STLGCGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISKDLMTDAELARAI SNMPTSAGQIKLMLENRAMVRRKGFGLIGVYGSSVIYMQLPFIG VIDTPCWIVKAAPSCSEKKNYACLLREDQGWYCKNAGSTVYY PNEKDCETRGDHFVCDTAAGINVAEQSKECNINISTNYPCKV SCGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQCFESIENSQA	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, N97G, P98G, R99G, Q100G, S101G, H368N, R102G	472
90	KESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDVENLTCD GPSLIKTELDTKSALRELRTVSADQLAREEQIEGGGGGGFVL GAIALGVATAAAVTAGVAIAKCIKRLSEVTAIKNALKKTNEAV STLGCGVRVLATAVRELKDFVSKNLTRAINKNKCDIPDLKMAV SFSQFNRRFLNVVRQFSDNAGITPAISKDLMTDAELARAI SNMPTSAGQIKLMLENRAMVRRKGFGLIGVYGSSVIYMQLPFIG VIDTPCWIVKAAPSCSEKKNYACLLREDQGWYCKNAGSTVYY PNEKDCETRGDHFVCDTAAGINVAEQSKECNINISTNYPCKV SCGRNPISMVALSPLGALVACYKGVSCSIGSNRVGIKQLNKG CSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRPVSSSFDPV KFPEDQFNVALDQCFESIENSQALVDQSNRILSSAEKGNTG	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, N97G, P98G, R99G, Q100G, S101G, H368N, R102G	490

[0133] Thus, in some embodiments, the hMPV F protein ectodomain shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NOs: 58-90, or an antigenic fragment thereof.

[0134] In some embodiments, the hMPV F protein comprises one or more furin cleavage sites, optionally one or more copies of an Arg-X-X-Arg motif (SEQ ID NO: 52), such as the

native sequence RQSR (SEQ ID NO: 54). Where a furin cleavage site is present in the hMPV ectodomain, expression yields may, in some cases, be increased by co-expression of furin, or functional variant thereof, from a transfected plasmid or from a stably integrated polynucleotide sequence in the host cell. In some embodiments, the furin cleavage site of hMPV F protein is modified by mutating the RQSR (SEQ ID NO: 54) motif to RRRR (SEQ ID NO: 55), or removed by substituting a Gly linker, or by making other amino acid substitutions.

[0135] hMPV neutralizing activity is mediated by antibodies recognizing both pre-fusion and post-fusion F protein conformations and antibodies generated in an *in vivo* immunological response can either selectively recognize pre-fusion specific or post-fusion specific sites, or can recognize both the pre-fusion and post-fusion forms of F protein. Battles et al. *Nat Commun.* 8:1528 (2017). The DS7, MPV196, MPV201, and MPV314 neutralizing antibodies bind an antigenic site accessible in both the pre-fusion and post-fusion forms of F protein, and is located on the DI and DII head domains of the F protein. Wen et al. *Nat Struct Mol Biol.* 19(4):461-463 (2012); Bar-Peled et al. *J Virol.* 93:e00342-19 (2019)). The MPE8 neutralizing antibody binds an epitope on F protein spanning the DI, DII, and DIII subunits, forming contacts dependent on the prefusion F conformation. Wen et al. *Nat Microbiol.* 2:16272 (2017). Several antigenic epitopes are shared with the respiratory syncytial virus (RSV) F protein, such as for example, the site III and site IV epitopes Huang et al. *Front Immunol.* 10:2778 (2019). These are examples and not an exhaustive list of epitopes described for neutralization antibodies against hMPV F protein.

[0136] The neutralizing epitopes are generally conformation epitopes so peptides corresponding to the epitopes do not bind the antibodies and thus do not induce neutralizing titers. Neutralizing antibodies can be isolated from healthy donors that are seropositive for hMPV, Battles et al., or can be generated by immunization in animal models, Gabriella et al. *J Virol.* 81(2):698-707 (2007).

[0137] In some embodiments, the VLPs of the disclosure comprising an hMPV F protein ectodomain can bind a neutralizing antibody against the hMPV F protein, also referred to as anti-hMPV F protein antibodies. In some embodiments, the VLPs described herein can bind anti-hMPV F protein antibodies known to selectively bind the prefusion form of hMPV F protein. Examples of antibodies that selectively bind the prefusion form of hMPV F protein include, without limitation, the MF10 and MPE8 antibodies. In some embodiments, the VLPs described herein bind anti-hMPV F protein antibodies known to selectively bind the postfusion

form of hMPV F protein. Examples of antibodies that selectively bind the postfusion form of hMPV F protein include, without limitation, the MF1, MF2, MF3, MF11, MF17, MF18, and MF19 antibodies. In some embodiments, the VLPs described herein can bind antibodies that bind both the prefusion and postfusion forms of hMPV F protein. Examples of antibodies that bind the prefusion and postfusion forms of hMPV F protein include, without limitation, the MF9, MF12, MF14, MF15, MF16, and MF20 antibodies. In some embodiments, the VLPs described herein bind one or more anti-hMPV F protein antibodies. In some embodiments, the VLPs described herein bind two or more anti-hMPV F protein antibodies.

Multimerization Domains and Linkers

[0138] In some embodiments, the VLP comprises a trimeric assembly of antigens comprising a first polypeptide comprising a first multimerization domain and a first polypeptide comprising a second multimerization domain. The first multimerization domain comprises a protein-protein interface that induces three copies of the first polypeptides to self-associate to form trimeric building blocks. In VLPs that have two or more components, each copy of the first multimerization domain further comprises a surface-exposed interface that interacts with a complementary surface-exposed interface on the second multimerization domain. Similarly stated, the second multimerization domain is adapted to multimerize with first multimerization domain (of the first polypeptide of the first component). 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 first multimerization domains and second multimerization domains drives the assembly of multiple copies of the trimeric assembly domain and second assembly domain into a target VLP. In some embodiments, each copy of the trimeric assembly domains of the VLP bears an antigenic protein, or antigenic fragment thereof, linked thereto (*e.g.*, as a genetic fusion); these VLPs display the proteins at full valency. In other embodiments, the VLPs of the disclosure comprise one or more copies of first multimerization domains bearing antigens proteins, or antigenic fragments thereof (*e.g.*, as genetic fusions) as well as one or more first multimerization domains that do not bear antigenic proteins; these VLPs display the G proteins at partial valency. The first multimerization domains can be any polypeptide sequence that forms a trimer and interacts with a second multimerization domains to drive assembly to a target VLP. In some embodiments, the VLP comprises first polypeptide and a second polypeptide selected from those disclosed in US 20130274441 A1, US 2015/0356240

A1, US 2016/0122392 A1, WO 2018/187325 A1, each of which is incorporated by reference herein in its entirety.

[0139] In some embodiments of the VLPs of the present disclosure, the antigenic protein and the core of the VLP may be genetically fused such that they are both present in a single polypeptide, also called a single chain polypeptide. The linkage between the protein and the core allows the antigenic protein, or antigenic fragment thereof, to be displayed on the exterior of the VLP. As such, the point of connection to the core should be on the exterior of the core of the virus-like particle formed. A wide variety of polypeptide sequences can be used to link the proteins, or antigenic fragments thereof and the core of the virus-like particle. In some cases the linker comprises that polypeptide sequence. Any suitable linker polypeptide can be used. In some embodiments, the linker imposes a rigid relative orientation of the antigenic protein (*e.g.* ectodomain) or antigenic fragment thereof to the core. In some embodiments, the linker flexibly links the antigenic protein (*e.g.* ectodomain) or antigenic fragment thereof to the core. In some embodiments, the linker includes additional trimerization domains (*e.g.*, the foldon domain of T4 fibrin) to assist in stabilizing the trimeric form of the F protein—*e.g.*, GYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO: 91) or a functional variant thereof.

[0140] In some embodiments, the linker may comprise a Gly-Ser linker (*i.e.* a linker consisting of glycine and serine residues) of any suitable length. In some 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 some embodiments, the Gly-Ser linker may comprise or consist of the amino acid sequence of GSGGSGSGSGSGSGSG (SEQ ID NO: 127), GGSGGSGS (SEQ ID NO: 128) or GSGGSGSG (SEQ ID NO: 129). In some embodiments, the linker comprises the sequence GSGSGSG (SEQ ID NO: 130). In some embodiments, the linker comprises the sequence GSGSGSGSGSGSGSGSG (SEQ ID NO: 131). In some embodiments, the linker comprises the sequence GSGGSGSGSGSGS (SEQ ID NO: 126).

In some embodiments of the VLPs of the present disclosure, the first component may optionally contain a poly-His tag, HHHHHH (SEQ ID NO: 143).

Table 3 shows illustrative examples of the VLP sequences described herein. The sequences comprise a multimerization domain, an hMPV F protein ectodomain, a linker, a poly-His tag, and a signal sequence. The signal and poly-His tag sequences are underlined and optionally included in the sequence.

Table 3. Illustrative Amino Acid Sequences of VLPs of the Disclosure

Construct name	SEQ ID NO:	Amino Acid Sequence
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hMPV001	92	MSWKVVII FSL LITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNVRLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLP FGVIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQAGSTVYYPNEKDCETR DHVFCDTAAGINVAEQSKECNINISTNYPCKVSTGRHPISMVALSPLGALVAC KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHV KGRPVSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILGSGGSGSGGSEK AAKAEAAARKMEELFKKHKIVAVLRANSVEEAI EKAVAVFAGGVHLIEITFTVPD ADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKE KGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFPVPTG GVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHH H
hMPV002	93	MSWKVVII FSL LITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNVRLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLP FGVIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQAGSTVYYPNEKDCETR DHVFCDTAAGINVAEQSKECNINISTNYPCKVSTGRHPISMVALSPLGALVAC KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHV KGRPVSSSFDPVKFPEDQFNVALDQVFESIENSQAGSGGSGSGGSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAI EKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV003	94	MSWKVVII FSL LITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNVRLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLP FGVIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQAGSTVYYPNEKDCETR DHVFCDTAAGINVAEQSKECNINISTNYPCKVSTGRHPISMVALSPLGALVAC KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHV KGRPVSSSFDPVKFPEDQFNVALDQVFESIENSQALVDQSNRILSSAEKNGTGGSGGS SGSGGSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAI EKAVAVFAGGVHL IEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHL DEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGP FPNVKFPVPTGGVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGA TELEHHHHHH
hMPV004	95	MSWKVVII FSL LITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLGNVRLATAVREL KDFVSKNLTRAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLP FGVIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQAGSTVYYPNEKDCETR DHVFCDTAAGINVAEQSKECNINISTNYPCKVSTGRHPISMVALSPLGALVAC KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHV KGRPVSSSFDPVKFPEDQFNVALDQVFESIENSQAGSGGSGSGGSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAI EKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV005	96	MSWKVVII FSL LITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKCI RLESEVTAIKNALKKTNEAVSTLGCGRVRLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLP FGVIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCQAGSTVYYPNEKDCETR DHVFCDTAAGINVAEQSKECNINISTNYPCKVSTGRNPISMVALSPLGALVAC

		KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPIKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV006	97	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCTDGPSLIKTELDTLSALRELKTCADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTTNECVSTLNGNVRVLATAVREL KEFVSKNLTSAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTAGQIKMLLENRCMVRRKGFILIGVYGSSSVIYMQLP FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNDKDCETRG DHVFCDTAAGINVAEQSRECNINISTTNPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPIKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV007	98	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCTDGPSLIKTELDTLSALRELKTCADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTTNECVSTLNGNVRVLATAVREL KEFVSKNLTSAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTAGQIKMLLENRCMVRRKGFILIGVYGSSSVIYMQLP FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNDKDCETRG DHVFCDTAAGINVAEQSRECNINISTTNPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPIKFPEDQFNVALDQVFESIENSQALVDQSNKILNSAESAI GSGSGS GSGGSEKAAKAEAAARKMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIE ITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEE ISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPN VKFVPTGGVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE LEHHHHHH
hMPV008	99	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCTDCPSLIKTELDTLSALRELKTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTTNECVSTLNGNVRVLATAVREL KEFVSKNLTSAINKNKCDIADLCMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTAGQIKMLLENRAMVRRKGFILIGVYGSSSVIYMQLP FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNDKDCETRG DHVFCDTAAGINVAEQSRECNINISTTNPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPICFPEDQFNVALDQVFESIENCQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV009	100	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCTDCPSLIKTELDTLSALRELKTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTTNECVSTLNGNVRVLATAVREL KEFVSKNLTSAINKNKCDIADLCMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTAGQIKMLLENRAMVRRKGFILIGVYGSSSVIYMQLP FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNDKDCETRG DHVFCDTAAGINVAEQSRECNINISTTNPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPICFPEDQFNVALDQVFESIENCQALVDQSNKILNSAESAI GSGSGS GSGGSEKAAKAEAAARKMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIE ITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEE ISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPN VKFVPTGGVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATE LEHHHHHH

hMPV010	101	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCSDCPSLIKTELDTKSALRELKTVSADQLAREEQIEGGGGGGFVLAIALG VATAAAVTAGVAIAKTI RLESEVTAIKNCLKTTNECVSTLGNGVRVLATAVRELK DFVSKNLTSAINKNKCDIDDLCAVSVFSQFNRRFLNVVRQFSDNAGITPAISLDL MTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLPFI GVIDTPCWIIKAAPSCSGKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRGD HVFCDTAAGINVAEQSKECNINISTTNYPCVKVSTGRHPISMVALSPLGALVACYK GVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRP VSSSFDPIKFPEDQFNVALDQVFENIENSQAGSGSGSGSGGSEKAAKAEAAARK MEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALSV LKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVM TPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKFVPTGGVNLNDNVAEW FKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV011	102	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCSDCPSLIKTELDTKSALRELKTVSADQLAREEQIEGGGGGGFVLAIALG VATAAAVTAGVAIAKTI RLESEVTAIKNCLKTTNECVSTLGNGVRVLATAVRELK DFVSKNLTSAINKNKCDIDDLCAVSVFSQFNRRFLNVVRQFSDNAGITPAISLDL MTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLPFI GVIDTPCWIIKAAPSCSGKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRGD HVFCDTAAGINVAEQSKECNINISTTNYPCVKVSTGRHPISMVALSPLGALVACYK GVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGRP VSSSFDPIKFPEDQFNVALDQVFENIENSQALVDQSNRISSAESAISSGSGSGS SGGSEKAAKAEAAARKMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEI TFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVM TPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKFVPTGGVNLNDNVAEW FKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV012	103	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCSDGPSLIKTELDTKSALRELKTVSADQLAREEQIENPRKARFVLGAIAL GVCTAAAVTCGIAIAKTI RLESEVNAIKGALKTTNEAVSTLGNGVRVLAFVREL KEFVSKNLTSAINKNKCDIADLKMAVSVFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLPFI FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNDKDCETR DHVFCDTACGINVAEQSRECNINISTTNYPCVKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYCLSKVEGEQHVIKGR PVSSSFDPIKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAARK MEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVM TPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKFVPTGGVNLNDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV013	104	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCSDGPSLIKTELDTKSALRELKTVSADQLAREEQIENPRKARFVLGAIAL GVCTAAAVTCGIAIAKTI RLESEVNAIKGALKTTNEAVSTLGNGVRVLAFVREL KEFVSKNLTSAINKNKCDIADLKMAVSVFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTSAGQIKMLLENRAMVRRKGFGLIGVYGSSVIYMQVLPFI FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNDKDCETR DHVFCDTACGINVAEQSRECNINISTTNYPCVKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYCLSKVEGEQHVIKGR PVSSSFDPIKFPEDQFNVALDQVFESIENSQALVDQSNKILNSAESAISSGSGSGS SGSGSEKAAKAEAAARKMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIE ITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVM TPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGFPPNVKFVPTGGVNLNDNVAEW FKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV014	105	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCSDGPSLIKTELDTKSALRELKTVSADQLAREEQIENPRRRRFLVLAIAL GVATAAAVTAGIAIAKTI RLESEVNAIKGCLKTTNECVSTLGNGVRVLATAVREL KEFVSKNLTSAINKNKCDIADLKMAVSVFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTSAGQIKMLLENRCMVRRKGFGLIGVYGSSVIYMQVLPFI FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNDKDCETR DHVFCDTAAGINVAEQSRECNINISTTNYPCVKVSTGRHPISMVALSPLGALVACY

		KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPIKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTILKLPFGEVVGPPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV015	106	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCTDCPSLIKTELDTKSALRELKTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGIAIAKTIRLESEVNAIKGCLKTTNECVSTLNGNVRVLATAVREL KEFVSKNLTSAINKNKCDIADLCMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTAGQIKLMLENRAMVRRKGFGILIGVYGSSSVIYMQVLP FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNKDCETRG DHVFCDTAAGINVAEQSRECNINISTNYPCKVSTGRHPI SMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPIKFPEDQFNVALDQVFESIENCQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTILKLPFGEVVGPPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV016	107	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCSDCPSLIKTELDTKSALRELKTVSADQLAREEQIENPRRRRFVLGAIALG VATAAAVTAGVAIAKTIRLESEVTAIKNCLKTTNECVSTLNGNVRVLATAVRELK DFVSKNLTSAINKNKCDIDDLCAVVSFSQFNRRFLNVVRQFSDNAGITPAISLDL MTDALARAVSNMPTAGQIKLMLENRAMVRRKGFGILIGVYGSSSVIYMQVLP FGVIDTPCWIIKAAPSCSGKKNYACLLREDQGWYCKNAGSTVYYPNKDCETRGD HVFCDTAAGINVAEQSKECNINISTNYPCKVSTGRHPI SMVALSPLGALVACYK GVSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPIKFPEDQFNVALDQVFENIENSQAGSGSGSGSGGSEKAAKAEAAAR MEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS LKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGVM TPTTELVKAMKLGHTILKLPFGEVVGPPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV017	108	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCTDGPSLIKTELDTKSALRELKTVSADQLAREEQIENPRRRRFVLGAIAL GVCTAAAVTCGIAIAKTIRLESEVNAIKGALKTTNEAVSTLNGNVRVLAFVREL KEFVSKNLTSALNKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTAGQIKLMLENRAMVRRKGFGILIGVYGSSSVIYMQVLP FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNKDCETRG DHVFCDTACGINVAEQSRECNINISTNYPCKVSTGRHPI SMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYCLSKVEGEQHVIGR PVSSSFDPIKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTILKLPFGEVVGPPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV018	109	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVCTAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLNGNVRVLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTAGQIKLMLENRAMVRRKGFGFLIGVYGSSSVIYMQVLP FGVIDTPCWIVKAAPSCSGKKNYACLLREDQGWYCKNAGSTVYYPNKDCETRG DHVFCDTACGINVAEQSKECNINISTNYPCKVSTGRHPI SMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPVKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTILKLPFGEVVGPPQFVKAMKGPFPNVKFPVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV019	110	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKTIRLESEVTAIKNALKKTNEAVSTLNGNVRVLAFVREL

		KDFVSKNLTRALNKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKLMLNENRAMVRRKGFGLIGVYSSSVIYMVQLPI FGVIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPVKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTIILKLPFGEVVGPPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV020	111	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCDGSPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVCTAAAVTAGVAIAKTIIRLESEVTAIKNALKKTNEAVSTLGNVGRVLAFAVREL KDFVSKNLTRALNKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKLMLNENRAMVRRKGFGLIGVYSSSVIYMVQLPI FGVIDTPCWIVKAAPSCSGKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTACGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPVKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTIILKLPFGEVVGPPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV021	112	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCTDCPSLIKTELDLTKSALRELKTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGIAIAKTIIRLESEVNAIKGALKTTNEAVSTLGNVGRVLAFAVREL KEFVSKNLTSAINKNKCDIADLCMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTSAGQIKLMLNENRAMVRRKGFGLIGVYSSSVIYMVQLPI FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCNAGSTVYYPNDKDCETRG DHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPVKFPEDQFNVALDQVFESIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTIILKLPFGEVVGPPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV022	113	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCTDCPSLIKTELDLTKSALRELKTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGIAIAKTIIRLESEVNAIKGALKTTNEAVSTLGNVGRVLAFAVREL KEFVSKNLTSAINKNKCDIADLCMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTSAGQIKLMLNENRAMVRRKGFGLIGVYSSSVIYMVQLPI FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCNAGSTVYYPNDKDCETRG DHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPKIAFPEDQFNVALDQVFESIENAQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTIILKLPFGEVVGPPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV023	114	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCDGSPSLIKTELDLTKSALRELKTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGVAIAKTIIRLESEVTAIKNALKKTNEAVSTLGNVGRVLAFAVREL KDFVSKNLTSAINKNKCDIDDLCAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKLMLNENRAMVRRKGFGLIGVYSSSVIYMVQLPI FGVIDTPCWIIKAAPSCSEKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNYPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPKIFPEDQFNVALDQVFENIENSQAGSGSGSGSGGSEKAAKAEAAAR KMEELFKKKHIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV

		MTPTTELVKAMKLGHTIILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV024	115	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADCPSLIKTELDLTKSALRELRTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGVAIAKTIKRLSEVTAIKNCLKKTNECVSTLGNVVRVLATAVREL KDFVSKNLTRAINKNKCDIADLCMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYSSSVIYMQLP FGVIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPVCFPEDQFNVALDQVFESIENCQAGSGSGSGSGSSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAIKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTTELVKAMKLGHTIILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV025	116	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADCPSLIKTELDLTKSALRELRTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGVAIAKTIKRLSEVTAIKNCLKKTNEAVSTLGNVVRVLATAVREL KDFVSKNLTRAINKNKCDIADLCMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSNMPTSAGQIKMLLENRAMVRRKGFGLIGVYSSSVIYMQLP FGVIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPVKFPEDQFNVALDQVFESIENSQAGSGSGSGSGSSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAIKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTTELVKAMKLGHTIILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV026	117	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKCIKRLSEVTAIKNCLKKTNEAVSTLGCGRVLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISKD LMTDAELARAI SNMPTSAGQIKMLLENRAMVRRKGFGLIGVYSSSVIYMQLP FGVIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNPCKVSCGRNPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPVKFPEDQFNVALDQCFESIENSQAGSGSGSGSGSSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAIKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTTELVKAMKLGHTIILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH
hMPV027	118	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKCIKRLSEVTAIKNCLKKTNEAVSTLGCGRVLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISKD LMTDAELARAI SNMPTSAGQIKMLLENRAMVRRKGFGLIGVYSSSVIYMQLP FGVIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNPCKVSCGRNPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIKGR PVSSSFDPVKFPEDQFNVALDQCFESIENSQALVDQSNRISSAEKNGTGGSGGS GSGSGSSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAIKAVAVFAGGVHL IEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHL DEEISQFAKEKGVFYMPGVMTPTTELVKAMKLGHTIILKLFPGEVVGPQFVKAMKGP FPNVKFVPTGGVNLDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGA TELEHHHHHH
hMPV028	119	MSWKVMIIISLLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADCPSLIKTELDLTKSALRELRTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGIAIAKTIKRLSEVNAIKGLKKTNECVSTLGNVVRVLATAVREL KEFVSKNLTSAINKNKCDIADLCMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTSAGQIKMLLENRAMVRRKGFGLIGVYSSSVIYMQLP FGVIDTPCWIIKAAPSCSEKDGNYACLLREDQGWYCKNAGSTVYYPNDKDCETRG

		DHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGKR PVSSSFDPICFPEDQFNVALDQVFESIENCQAGSGSGSGSGSGGSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGV MTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGCTELEHHHHHH
hMPV029	120	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKCIIRLESEVTAIKNALKKTNEAVSTLGCGRVRLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISKD LMTDAELARAI SNMPTSAGQIKLMLENRAMVRRKGFILIGVYSSSVIYMQVLP FGVIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNYPCKVSCGRNPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGKR PVSSSFDPVKFPEDQFNVALDQCFESIENSQAGSGSGSGSGSGGSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGV MTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGCTELEHHHHHH
hMPV030	121	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDLTKSALRELRTVSADQLAREEQIENPRRRRFVLGAIAL GVATAAAVTAGVAIAKCIIRLESEVTAIKNALKKTNEAVSTLGCGRVRLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISKD LMTDAELARAI SNMPTSAGQIKLMLENRAMVRRKGFILIGVYSSSVIYMQVLP FGVIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNYPCKVSCGRNPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGKR PVSSSFDPVKFPEDQFNVALDQCFESIENSQALVDQSNRISSAEKNGTGGSGGS SGSGSGSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHL IEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHL DEEISQFCKEKGVFYMPGVMTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGP FPNVKFVPTGGVNLDNVCEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRG CTELEHHHHHH
hMPV031	122	MSWKVMIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDLTKSALRELKTCADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGIAIAKTIIRLESEVNAIKGCLKTTNECVSTLGNVRLATAVREL KEFVSKNLTSAINKNKCDIADLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISLD LMTDAELARAVSYMPTSAGQIKLMLENRCMVRRKGFILIGVYSSSVIYMQVLP FGVIDTPCWIIKAAPSCSEKDGNACLLREDQGWYCKNAGSTVYYPNDKDCETRG DHVFCDTAAGINVAEQSRECNINISTTNYPCKVSTGRHPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLPKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGKR PVSSSFDPIKFPEDQFNVALDQVFESIENSQAGSGSGSGSGSGGSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQCRKAVESGAEFIVSPHLDEEISQFCKEKGVFYMPGV MTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVCE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGCTELEHHHHHH
hMPV033	123	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDLTKSALRELRTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGVAIAKCIIRLESEVTAIKNALKKTNEAVSTLGCGRVRLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISKD LMTDAELARAI SNMPTSAGQIKLMLENRAMVRRKGFILIGVYSSSVIYMQVLP FGVIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCNAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTTNYPCKVSCGRNPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGKR PVSSSFDPVKFPEDQFNVALDQCFESIENSQAGSGSGSGSGSGGSEKAAKAEAAAR KMEELFKKHKIVAVLRANSVEEAIEKAVAVFAGGVHLIEITFTVPDADTVIKALS VLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLDEEISQFAKEKGVFYMPGV MTPTELVKAMKLGHTILKLFPGEVVGPQFVKAMKGPFPNVKFVPTGGVNLDNVAE WFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGATELEHHHHHH

hMPV034	124	MSWKVVIIIFSLITPQHGLKESYLEESCSTITEGYLSVLRTGWYTNVFTLEVGDV ENLTCADGPSLIKTELDTKSALRELRTVSADQLAREEQIEGGGGGGFVLGAIAL GVATAAAVTAGVAIAKCI RLESEVTAIKNALKKTNEAVSTLGCGRVRLATAVREL KDFVSKNLTRAINKNKCDIPDLKMAVSFSQFNRRFLNVVRQFSDNAGITPAISKD LMTDAELARAI SNMPTSAGQIKLMLENRAMVRRKGFILIGVYGSSVIYMQLP FGVIDTPCWIVKAAPSCSEKKGNACLLREDQGWYCQONAGSTVYYPNEKDCETRG DHVFCDTAAGINVAEQSKECNINISTNYPCKVSCGRNPISMVALSPLGALVACY KGVSCSIGSNRVGIIKQLNKGCSYITNQDADTVTIDNTVYQLSKVEGEQHVIGR PVSSSFDPVKFPEDQFNVALDQCFESIENSQALVDQSNRILSSAEKGNTGGSGGS GSGSGGSEKAAKAEAAARKMEELFKKHKIVAVLRANSVEEAEKAVAVFAGGVHL IEITFTVPDADTVIKALSVLKEKGAIIGAGTVTSVEQARKAVESGAEFIVSPHLD EEISQFAKEKGVFYMPGVMPTTELVKAMKLIGHTILKLFPGEVVGPQFVKAMKGP PNVKFVPTGGVNLNDNVAEWFKAGVLAVGVGSALVKGTPDEVREKAKAFVEKIRGA TELEHHHHHH
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Assembly of VLPs

[0141] In some embodiments, a single component self-assembles into the VLP. In some embodiments, one or more purified samples of first and second components for use in forming a VLP are mixed in an approximately equimolar molar ratio in aqueous conditions (*e.g.*, an I53-50A/B isosahedral VLP). The first and second components (through the multimerization domains and optionally through the ectodomains) interact with one another to drive assembly of the target VLP. Successful assembly of the target VLP 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 VLP can be purified from other species or molecules present in the *in vitro* assembly reaction using preparative techniques commonly 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 antigenic protein in the VLP 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, ELISA, surface plasmon resonance, biolayer interferometry, or amino acid analysis. The accessibility of the 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, surface plasmon resonance, biolayer interferometry, or antisera specific to the antigen.

[0142] In various embodiments, the VLPs of the disclosure comprise two or more distinct first polypeptides bearing different antigenic proteins as genetic fusions; these VLPs co-display multiple different proteins on the same VLP. These multi-antigen VLPs are produced by performing *in vitro* assembly with mixtures of two or more antigens each comprising a multimerization domain. The fraction of each antigen in the mixture determines the average valency of each antigenic protein in the resulting VLPs. The presence and average valency of each antigen in a given sample can be assessed by quantitative analysis using the techniques described above for evaluating the presence of antigenic proteins in full-valency VLPs.

[0143] In various embodiments, the VLPs 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.

[0144] In some embodiments, the VLPs has icosahedral symmetry. In such embodiment, the VLP may comprise 60 copies of a first component and 60 copies of a second component. In one such embodiment, the number of identical first polypeptides in each first assembly is different than the number of identical first polypeptides in each second assembly. For example, in some embodiments, the VLP comprises twelve first assemblies and twenty second assemblies; in such embodiments, each first assembly may, for example, comprise five copies of the identical first component, and each second assembly may, for example, comprise three copies of the identical second component. In other embodiments, the VLP comprises twelve first assemblies and thirty second assemblies; in such an embodiment, each first assembly may, for example, comprise five copies of the identical first component, and each second assembly may, for example, comprise two copies of the identical second component. In further embodiments, the VLP comprises twenty first assemblies and thirty second assemblies; in this embodiment, each first assembly may, for example, comprise three copies of the identical first component, and each second assembly may, for example, comprise two copies of the identical second component. All of these embodiments are capable of forming protein-based VLPs with regular icosahedral symmetry.

[0145] In various further embodiments, oligomeric states of the first and second multimerization domains are as follows:

I53-34A: trimer + I53-34B: pentamer;

I53-40A: pentamer + I53-40B: trimer;

I53-47A: trimer + I53-47B: pentamer;

I53-50A: trimer + I53-50B: pentamer;

I53-51A: trimer + I53-51B: pentamer;
 I32-06A: dimer + I32-06B: trimer;
 I32-19A: trimer + I32-19B: dimer;
 I32-28A: trimer + I32-28B: dimer;
 I52-03A: pentamer + I52-03B: dimer;
 I52-32A: dimer + I52-32B: pentamer; and
 I52-33A: pentamer + I52-33B: dimer

[0146] In some embodiments, the second multimerization domain of the second polypeptide comprises a sequence that shares at least 95% identity to I53-50A or a variant thereof:

I53-50A

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      1 MEELFKKHKI VAVLRANSVE EAIEKAVAVF AGGVHLIEIT
     41 FTVPDADTVI KALSVLKEKG AIIGAGTVTS VEQCRKAVES
     81 GAEFIVSPHL DEEISQFCKE KGVFYMPGVM TPTELVKAMK
    121 LGHTILKLFP GEVVGPFQVK AMKGPFPNVK FVPTGGVNLD
    161 NVCEWFKAGV LAVGVGSALV KGTPDEVREK AKAFVEKIRG
    201 CTE
  (SEQ ID NO: 144)
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[0147] In some embodiments, the second multimerization domain shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 7 or SEQ ID NO: 144, or an antigenic fragment thereof.

[0148] The I53-50A protein sequence has two intra-monomer disulfide bonds. In some embodiments, the cysteine residues are mutated to residues that do not contain a thiol group (*e.g.* alanine or serine). Removal of the thiol group may promote correct protein folding while not impairing multimerization. In some embodiments, the multimerization domain of the first polypeptide comprises an amino acid substitution at one or more of positions 74, 98, 163, and 201 relative to SEQ ID NO: 144, as shown here:

I53-50A

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      1 MEELFKKHKI VAVLRANSVE EAIEKAVAVF AGGVHLIEIT
     41 FTVPDADTVI KALSVLKEKG AIIGAGTVTS VEQCRKAVES
     81 GAEFIVSPHL DEEISQFCKE KGVFYMPGVM TPTELVKAMK
    121 LGHTILKLFP GEVVGPFQVK AMKGPFPNVK FVPTGGVNLD
    161 NVCEWFKAGV LAVGVGSALV KGTPDEVREK AKAFVEKIRG
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201 CTE
144)

(SEQ ID NO:

[0149] In some embodiments, the multimerization domain of the first polypeptide comprises an amino acid substitution of one or more of C74A, C98A, C163A, and C201A relative to SEQ ID NO: 132. In some embodiments, the multimerization domain of the first polypeptide comprises SEQ ID NO: 132 or a variant thereof.

I53-50A-Δcys (disulfide-free form)

1 MEELFKKHKI VAVLRANSVE EAIEKAVAVF AGGVHLIEIT
41 FTVPDADTVI KALSVLKEKG AIIGAGTVTS VEQAARKAVES
81 GAEFIVSPHL DEEISQFAKE KGVFYMPGVM TPTELVKAMK
121 LGHTILKLFP GEVVGPFQFVK AMKGPFPNVK FVPTGGVNLN
161 NVAEWFKAGV LAVGVGSALV KGTPDEVREK AKAFVEKIRG
201 ATE (SEQ ID NO:
132)

[0150] In some embodiments, the multimerization domain shares at least 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to SEQ ID NO: 144 or SEQ ID NO: 132, or an antigenic fragment thereof, and comprises one, two, three or four amino acid substitutions selected from C74A, C98A, C163A, and C201A. Alternatively, the substitution may be of C to A, T, S, L, I, or any amino acid other than C.

Nucleic Acids

[0151] In another aspect, the present disclosure provides isolated nucleic acids encoding an antigen, a first component, and/or a second component, of the present disclosure. 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 disclosure.

[0152] In a further aspect, the present disclosure provides recombinant expression vectors comprising the isolated nucleic acid of any embodiment or combination of embodiments of the disclosure operatively linked 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 disclosure 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 disclosure is intended to include other expression vectors that serve equivalent functions, such as viral vectors.

[0153] In another aspect, the present disclosure provides host cells that have been transfected or transduced with the recombinant expression vectors disclosed herein, wherein the host cells can be either prokaryotic or eukaryotic. The cells can be transiently or stably transfected or transduced. Such transfection or transduction 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).

[0154] In another aspect, the disclosure provides a method of producing an antigen, component, or VLP according to the disclosure. In some embodiments, the method comprises the steps of (a) culturing a host according to this aspect of the disclosure under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide.

[0155] In some embodiments, the disclosure provides a method of manufacturing a vaccine, comprising culturing a host cell comprising a polynucleotide comprising a sequence encoding the antigen of the disclosure in a culture medium so that the host cell secretes the antigen into the culture media; optionally purifying the antigen from the culture media; mixing the antigen with a second component, wherein the second component multimerizes with the antigen to form a VLP; and optionally purifying the VLP.

[0156] In some embodiments, the disclosure provides method of manufacturing a vaccine, comprising culturing a host cell comprising one or more polynucleotides comprising sequences encoding both components of the VLP of any one of disclosure so that the host cell secretes the first component and the second component into the culture media; and optionally purifying the VLP from the culture media.

[0157] Illustrative host cells include E. coli cells, 293 and 293F cells, HEK293 cells, Sf9 cells, Chinese hamster ovary (CHO) cells and any other cell line used in the production of recombinant proteins.

[0158] In various embodiments, the first component expresses at about 0.5 mg/mL, about 1.0 mg/mL, about 1.5 mg/mL, about 2.5 mg/mL, about 5 mg/mL, about 10 mg/mL, about 25 mg/mL, about 50 mg/mL, about 75 mg/mL, about 100 mg/mL, or greater in a method of manufacturing according to the disclosure (e.g. 293F cells grown in suspension). In various embodiments, the first component expresses at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of the expression level of a hMPV F protein (optionally the same ectodomain as in the VLP) in the same or similar expression system. In various embodiments, the first component expresses at least 105%, at least 110%, at least 115%, at

least 120%, at least 125%, at least 150%, at least 175%, or at least 200% of the expression level of a hMPV F protein (optionally the same ectodomain as in the VLP) in the same or similar expression system.

[0159] In some embodiments, the hMPV F protein ectodomain of the first component is in the prefusion conformation, or a substantial fraction of the hMPV F protein ectodomain is in the prefusion conformation. In various embodiments, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of the hMPV F protein ectodomain of the first component is in the prefusion conformation. In some embodiments, the hMPV F protein ectodomain of the VLP is in the prefusion conformation, or a substantial fraction of the hMPV F protein ectodomain is in the prefusion conformation. In various embodiments, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95% of the hMPV F protein ectodomain of the VLP is in the prefusion conformation.

[0160] In some embodiments, the fraction of hMPV F protein ectodomain in the prefusion state is determined by binding to a conformation-specific antibody (*e.g.*, D1-25 and 1112-1). In some embodiments, the fraction of hMPV F protein ectodomain in the first component in the prefusion conformation is at least 105%, at least 110%, at least 115%, at least 120%, at least 125%, at least 150%, at least 175%, or at least 200% greater than the fraction in a reference component, or than the fraction in a reference protein not linked to a multimerization domain. In some embodiments, the fraction of hMPV F protein ectodomain in the first component in the prefusion conformation is at least 105%, at least 110%, at least 115%, at least 120%, at least 125%, at least 150%, at least 175%, or at least 200% greater than the fraction in a VLP (*e.g.*, micelle VLP)

Vaccines and Administration

[0161] The disclosure also provides vaccines comprising the VLPs described herein. Such compositions can be used to raise antibodies in a mammal (*e.g.* a human). The vaccines compositions of the disclosure typically include a pharmaceutically acceptable carrier, and a thorough discussion of such carriers is available in Remington: The Science and Practice of Pharmacy.

[0162] The pH of the composition is usually between about 4.5 to about 11, such as between about 5 to about 11, between about 5.5 to about 11, between about 6 to about 11, between about 5 to about 10.5, between about 5.5 to about 10.5, between about 6 to about 10.5, between about 5 to about 10, between about 5.5 to about 10, between about 6 to about 10,

between about 5 to about 9.5, between about 5.5 to about 9.5, between about 6 to about 9.5, between about 5 to about 9, between about 5.5 to about 9, between about 6 to about 9, between about 5 to about 8.5, between about 5.5 to about 8.5, between about 6 to about 8.5, between about 5 to about 8, between about 5.5 to about 8, between about 6 to about 8, about 4.5, about 5, about 6.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10, about 10.5, about 11, etc. Stable pH may be maintained by the use of a buffer e.g. a Tris buffer, a citrate buffer, phosphate buffer, or a histidine buffer. Thus a composition will generally include a buffer.

[0163] A composition may be sterile and/or pyrogen free. Compositions may be isotonic with respect to humans.

[0164] A vaccine composition comprises an immunologically effective amount of its antigen(s). An “immunologically effective amount” or “effective amount” is an amount which, when administered to a subject, is effective for eliciting an antibody response against the antigen. This amount can vary depending upon the health and physical condition of the individual to be treated, their age, the capacity of the individual's immune system to synthesize antibodies, the degree of protection desired, the formulation of the vaccine, the treating doctor's assessment of the medical situation, and other relevant factors. It is expected that the amount will fall in a relatively broad range that can be determined through routine trials. The antigen content of compositions of the disclosure will generally be expressed in terms of the mass of protein per dose. A dose of 10-500 μ g (e.g. 50 μ g) per antigen can be useful.

[0165] Vaccine compositions may include an immunological adjuvant. Illustrative adjuvants include the following: 1. mineral-containing compositions; 2. oil emulsions; 3. saponin formulations; 4. virosomes and virus-like particles; 5. bacterial or microbial derivatives; 6. bioadhesives and mucoadhesives; 7. liposomes; 8. polyoxyethylene ether and polyoxyethylene ester formulations; 9. polyphosphazene (pcpp); 10. muramyl peptides; 11. imidazoquinolone compounds; 12. thiosemicarbazone compounds; 13. tryptanthrin compounds; 14. human immunomodulators; 15. lipopeptides; 16. benzonaphthyridines; 17. microparticles; 18. immunostimulatory polynucleotide (such as rna or dna; e.g., cpg-containing oligonucleotides).

[0166] For example, the composition may include an aluminum salt adjuvant, an oil in water emulsion (e.g. an oil-in-water emulsion comprising squalene, such as MF59 or AS03), a TLR7 agonist (such as imidazoquinoline or imiquimod), or a combination thereof. Suitable aluminum salts include hydroxides (e.g. oxyhydroxides), phosphates (e.g. hydroxyphosphates,

orthophosphates), (e.g. see chapters 8 & 9 of Vaccine Design. (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum), or mixtures thereof. The salts can take any suitable form (e.g. gel, crystalline, amorphous, etc.), with adsorption of antigen to the salt being an example. The concentration of Al^{+++} in a composition for administration to a patient may be less than 5mg/ml e.g. <4 mg/ml, <3 mg/ml, <2 mg/ml, <1 mg/ml, etc. A preferred range is between 0.3 and 1 mg/ml. A maximum of 0.85mg/dose is preferred. Aluminum hydroxide and aluminium phosphate adjuvants are suitable for use with the disclosure.

[0167] Exemplary adjuvants that may be used in a pharmaceutical composition provided herein include, but are not limited to, 3M-052, Adju-PhosTM, AlhydrogelTM, AdjumerTM, albumin-heparin microparticles, Algal Glucan, Algammulin, Alum, Antigen Formulation, AS-2 adjuvant, ASO1, ASO3, autologous dendritic cells, autologous PBMC, AvridineTM, B7-2, BAK, BAY R1005, BECC TLR-4 agonists, 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, CPG-1018, CRL1005, Cytokine-containing Liposomes, D-Murapalmitine, 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/GMCSF plasmid (Sykes), IL-2 in pcDNA3, IL-2/Ig plasmid, IL-2/Ig protein, IL-4, IL-4 in pcDNA3, ImiquimodTM, ImmTherTM, Immunoliposomes Containing Antibodies to Costimulatory Molecules, Interferon-gamma, Interleukin-1 beta, Interleukin-12, Interleukin-2, Interleukin-7, ISCOM(s)TM, Iscopen 7.0.3TM, Keyhole Limpet Hemocyanin, Lipid-based Adjuvant, Liposomes, Loxoribine, LT(R192G), LT-OA or LT Oral Adjuvant, LT-R192G, LTK63, LTK72, Matrix-MTM adjuvant, MF59, MONTANIDE ISA 51, MONTANIDE ISA 720, MPLTM, MPL-SE, MTP-PE, MTP-PE Liposomes, Murametide, Murapalmitine, 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, pCMVmCAT1, 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, Ribi like 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, SWE, Tetanus

toxoid (TT), Theramide™, Threonyl muramyl dipeptide (TMDP), Ty Particles, and Walter Reed Liposomes.

[0168] In preferred embodiments, the adjuvant is an aluminium hydroxide gel (e.g., Alhydrogel™). In preferred embodiments, the adjuvant is SWE. In preferred embodiments, the adjuvant is MF59.

[0169] MF59 is an oil-in-water emulsion containing squalene (4.3%) in citric acid buffer with stabilizing nonionic surfactants Tween 80 (0.5%) and Span 85 (0.5%). MF59 has been shown to be well-tolerated in humans and is used in vaccines against seasonal influenza (see Ko and Kang, *Hum Vaccin Immunother.* 2018; 14(12): 3041–3045; U.S. Patent No. 6,299,884).

[0170] For example, the composition may include an aluminum salt adjuvant, an oil in water emulsion (e.g. an oil-in-water emulsion comprising squalene, such as MF59, SWE, or AS03), a TLR9 agonist (such as CpG oligodeoxynucleotides), a TLR7 agonist (such as imidazoquinoline or imiquimod), or a combination thereof. In some embodiments, the adjuvant is a combination of an aluminum salt and CPG1018. Suitable aluminum salts include hydroxides (e.g. oxyhydroxides), phosphates (e.g. hydroxyphosphates, orthophosphates), (e.g. see chapters 8 & 9 of *Vaccine Design.* (1995) eds. Powell & Newman. ISBN: 030644867X. Plenum), or mixtures thereof. The salts can take any suitable form (e.g. gel, crystalline, amorphous, etc.), with adsorption of antigen to the salt being an example. The concentration of Al+++ in a composition for administration to a patient may be less than 5mg/ml e.g. <4 mg/ml, <3 mg/ml, <2 mg/ml, <1 mg/ml, etc. A preferred range is between 0.3 and 1 mg/ml. A maximum of 0.85mg/dose is preferred. Aluminum hydroxide and aluminium phosphate adjuvants are suitable for use with the disclosure. In a preferred embodiment, a pharmaceutical composition provided herein comprises aluminum hydroxide as an adjuvant. In some embodiment, a pharmaceutical composition provided herein comprises 500 µg aluminium hydroxide.

[0171] Selection of an adjuvant depends on the subject to be treated. Preferably, a pharmaceutically acceptable adjuvant is used.

[0172] In some embodiments, the adjuvant is a squalene emulsion.

[0173] In some embodiments, the adjuvant is a TLR4 immunostimulant (e.g., SLA, GLA), e.g., as described in Van Hoeven et al. *PLoS One.* 11(2):e0149610 (2016).

[0174] In some embodiments, the adjuvant is a TLR7/8 immunostimulant (e.g., R848, IMQ, 3M-052), e.g., as described in Dowling D. *ImmunoHorizons* (6):185-197 (2018).

[0175] In some embodiments, the adjuvant is a TLR9 immunostimulant (CpG), *e.g.*, as described in Bode et al. *Expert Rev Vaccines*. 10(4):499–511 (2011).

[0176] In some embodiments, the adjuvant is saponin (QS21), *e.g.*, as described in Zhu et al. *Nat Prod Chem Res*. 3(4):e113 (2016).

[0177] In some embodiments, the vaccine comprises a combination of two or more adjuvants (*e.g.* squalene emulsion and alum or a TLR4 immunostimulant).

[0178] One suitable immunological adjuvant comprises a compound of Formula (I) as defined in WO2011/027222, or a pharmaceutically acceptable salt thereof, adsorbed to an aluminum salt. Many further adjuvants can be used, including any of those disclosed in Powell & Newman (1995).

[0179] Compositions may include an antimicrobial, particularly when packaged in multiple dose format. Antimicrobials such as thiomersal and 2-phenoxyethanol are commonly found in vaccines, but sometimes it may be desirable to use either a mercury-free preservative or no preservative at all.

[0180] Compositions may comprise detergent *e.g.* a polysorbate, such as polysorbate 80. Detergents are generally present at low levels *e.g.* <0.01%.

[0181] Compositions may include sodium salts (*e.g.* sodium chloride) to give tonicity. A concentration of 10±2 mg/ml NaCl is typical *e.g.* about 9 mg/ml.

[0182] In some embodiments, the buffer in the vaccine 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 trioleate, 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. Illustrative 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 VLP, in lyophilized or liquid form. Illustrative stabilizers include sucrose, sorbitol, glycine, inositol, sodium chloride, methionine, arginine, and arginine hydrochloride.

[0183] In some embodiments, the disclosure provides a vaccine (immunogenic composition) comprising one or more pharmaceutically acceptable excipients.

[0184] In some embodiments, the vaccine (immunogenic composition) is a stable emulsion.

[0185] In some embodiments, the disclosure provides a vaccine (immunogenic composition) comprises one or more adjuvants. In some embodiments, the one or more adjuvants comprises a TLR4 immunostimulant, e.g., Monophosphoryl Lipid A (MPL), Glucopyranosyl Lipid A (GLA), and/or Soluble *Leishmania* Antigen (SLA).

[0186] In another aspect, the disclosure provides a method of inducing an immune response against hMPV, comprising administering to a subject in need thereof an immunologically effective amount of the immunogenic composition described herein, which comprises the VLP as described herein.

[0187] In certain embodiments, the immune response comprises the production of neutralizing antibodies against an infectious agent. In certain embodiments, the neutralizing antibodies are complement-independent.

[0188] The immune response can comprise a humoral immune response, a cell-mediated immune response, or both. In some embodiments an immune response is induced against each delivered antigenic protein. A cell-mediated immune response can comprise a Helper T-cell (Th) response, a CD8+ cytotoxic T-cell (CTL) response, or both. In some embodiments the immune response comprises a humoral immune response, and the antibodies are neutralizing antibodies. Neutralizing antibodies block viral infection of cells. Viruses infect epithelial cells and also fibroblast cells. In some embodiments the immune response reduces or prevents infection of both cell types. Neutralizing antibody responses can be complement-dependent or complement-independent. In some embodiments the neutralizing antibody response is complement-independent. In some embodiments the neutralizing antibody response is cross-neutralizing; i.e., an antibody generated against an administered composition neutralizes a virus of a strain other than the strain used in the composition.

[0189] A useful measure of antibody potency in the art is “50% neutralization titer.” To determine 50% neutralizing titer, serum from immunized animals is diluted to assess how dilute

serum can be yet retain the ability to block entry of 50% of viruses into cells. For example, a titer of 700 means that serum retained the ability to neutralize 50% of virus after being diluted 700-fold. Thus, higher titers indicate more potent neutralizing antibody responses. In some embodiments, this titer is in a range having a lower limit of about 200, about 400, about 600, about 800, about 1000, about 1500, about 2000, about 2500, about 3000, about 3500, about 4000, about 4500, about 5000, about 5500, about 6000, about 6500, or about 7000. The 50% neutralization titer range can have an upper limit of about 400, about 600, about 800, about 1000, about 1500, about 2000, about 2500, about 3000, about 3500, about 4000, about 4500, about 5000, about 5500, about 6000, about 6500, about 7000, about 8000, about 9000, about 10000, about 11000, about 12000, about 13000, about 14000, about 15000, about 16000, about 17000, about 18000, about 19000, about 20000, about 21000, about 22000, about 23000, about 24000, about 25000, about 26000, about 27000, about 28000, about 29000, or about 30000. For example, the 50% neutralization titer can be about 3000 to about 25000. "About" means plus or minus 10% of the recited value.

[0190] In some embodiments, the virus-like particles of the disclosure generate an immune response of 0.5 IU/mL, 1.0 IU/mL, 1.5 IU/mL, 2.0 IU/mL, 10 IU/mL, 25 IU/mL, 50 IU/mL, 100 IU/mL or greater. In some embodiments, the virus-like particles of the disclosure generate an immune response of 0.5 IU/mL or greater.

[0191] Compositions of the disclosure will generally be administered directly to a subject. Direct delivery may be accomplished by parenteral injection (*e.g.* subcutaneously, intraperitoneally, intravenously, intramuscularly, or to the interstitial space of a tissue), orally, intranasal, or by any other suitable route. For example, intramuscular administration may be used *e.g.* to the thigh or the upper arm. Injection may be via a needle (*e.g.* a hypodermic needle), but needle-free injection may alternatively be used. A typical intramuscular dosage volume is 0.5 ml.

[0192] Dosage can be by a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunization schedule and/or in a booster immunization schedule. In a multiple dose schedule the various doses may be given by the same or different routes, *e.g.*, a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, etc. Multiple doses will typically be administered at least 1 week apart (*e.g.*, about 2 weeks, about 3 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 10 weeks, about 12 weeks, about 16 weeks, etc.). Multiple doses may be administered at least 1 month apart (*e.g.*, about 2 months, about 3 months, about 4 months, about 6 months, about 8 months, about 10 months, about 12 months,

about 16 months, etc.). A second or subsequent dose may be administered over longer intervals, *e.g.*, about 1 year or about 2 years after the previous dose.

[0193] Where the vaccine is for prophylactic use, the human is preferably a child (*e.g.*, a toddler or infant), a teenager, or an adult; where the vaccine is for therapeutic use, the human is preferably a teenager or an adult. A vaccine intended for children may also be administered to adults, *e.g.*, to assess safety, dosage, immunogenicity, etc.

[0194] Vaccines of the disclosure may be prophylactic (*i.e.* to prevent disease) or therapeutic (*i.e.* to reduce or eliminate the symptoms of a disease). The term prophylactic may be considered as reducing the severity of or preventing the onset of a particular condition. For the avoidance of doubt, the term prophylactic vaccine may also refer to vaccines that ameliorate the effects of a future infection, for example by reducing the severity or duration of such an infection.

[0195] Isolated and/or purified VLPs described herein can be administered alone or as either prime or boost in mixed-modality regimes, such as a RNA prime or DNA primer followed by a protein boost. Benefits of the RNA-prime/protein-boost strategy, as compared to a protein-prime/protein-boost strategy, include, for example, increased antibody titers, a more balanced IgG1:IgG2a subtype profile, induction of TH1-type CD4⁺ T cell-mediated immune response that was similar to that of viral particles, and reduced production of non-neutralizing antibodies. The RNA prime can increase the immunogenicity of compositions regardless of whether they contain or do not contain an adjuvant.

[0196] In the RNA-prime/protein boost-strategy, the RNA and the protein are directed to the same target antigen. Examples of suitable modes of delivering RNAs include virus-like replicon particles (VRPs), alphavirus RNA, replicons encapsulated in lipid nanoparticles (LNPs) or formulated RNAs, such as replicons formulated with cationic nanoemulsions (CNEs). Suitable cationic oil-in-water nanoemulsions are disclosed in WO2012/006380 *e.g.* comprising an oil core (*e.g.* comprising squalene) and a cationic lipid (*e.g.* DOTAP, DMTAP, DSTAP, DC-cholesterol, etc.).

[0197] Alternatively, two doses of the VLP may be administered at a predetermined interval to achieve a prime-boost effect. The predetermined interval may be 1, 2, 3, 4, 6, 7, 10, or 14 days; or 3-5 days, 7-10 days, or 10-14 days or the like. The predetermined interval may be 1, 2, 3, 4, or 6 weeks; or 2-3 weeks, 3-4 weeks, or 5-6 weeks or the like. The predetermined interval may be 1, 2, 3, or 4 months.

[0198] In some embodiments, the RNA molecule is encapsulated in, bound to or adsorbed on a cationic lipid, a liposome, a cochleate, a virosome, an immune-stimulating complex, a microparticle, a microsphere, a nanosphere, a unilamellar vesicle, a multilamellar vesicle, an oil-in-water emulsion, a water-in-oil emulsion, an emulsome, a polycationic peptide, a cationic nanoemulsion, or combinations thereof.

[0199] The disclosure further provides combination vaccines. The vaccines of the disclosure include vaccines comprising both a hMPV VLP and vaccines for one or more of: SARS-CoV-2, respiratory syncytial virus, Rabies, Typhoid fever, Hepatitis A, Polio, Influenza, Hepatitis B, Yellow Fever, Japanese encephalitis, Parvovirus, Distemper, Adenovirus, Parainfluenza, Influenza, Measles, Lyme disease, Coronavirus, Vesicular stomatitis virus, Herpes simplex virus, Baculovirus, Thogotovirus, and Bornaviridae.

[0200] Also provided herein are kits for administration of nucleic acid (e.g., RNA), purified proteins, and purified VLPs described herein, and instructions for use. The disclosure also provides a delivery device pre-filled with a composition or a vaccine disclosed herein.

[0201] The pharmaceutical compositions described herein can be administered in combination with one or more additional therapeutic agents. The additional therapeutic agents may include, but are not limited to antibiotics or antibacterial agents, antiemetic agents, antifungal agents, anti-inflammatory agents, antiviral agents, immunomodulatory agents, cytokines, antidepressants, hormones, alkylating agents, antimetabolites, antitumour antibiotics, antimitotic agents, topoisomerase inhibitors, cytostatic agents, anti-invasion agents, antiangiogenic agents, inhibitors of growth factor function inhibitors of viral replication, viral enzyme inhibitors, anticancer agents, α -interferons, β -interferon, ribavirin, hormones, and other toll-like receptor modulators, immunoglobulins (Igs), and antibodies modulating Ig function (such as anti-IgE (omalizumab)).

[0202] In certain embodiments, the compositions disclosed herein may be used as a medicament, e.g., for use in inducing or enhancing an immune response in a subject in need thereof, such as a mammal.

[0203] In certain embodiments, the compositions disclosed herein may be used in the manufacture of a medicament for inducing or enhancing an immune response in a subject in need thereof, such as a mammal.

[0204] One way of checking efficacy of therapeutic treatment involves monitoring infection by an infectious agent after administration of the compositions or vaccines disclosed herein. One way of checking efficacy of prophylactic treatment involves monitoring immune

responses, systemically (such as monitoring the level of IgG1 and IgG2a production) and/or mucosally (such as monitoring the level of IgA production), against the antigen. Typically, antigen-specific serum antibody responses are determined post-immunization but pre-challenge whereas antigen-specific mucosal antibody responses are determined post-immunization and post-challenge.

EXAMPLES

Example 1

[0205] This example illustrates production of a two-component, icosahedral VLP intended for use as a vaccine for hMPV virus. As first component A, the vaccine uses a polypeptide antigen composed of the ectodomain of hMPV F protein, C-terminally fused to the I53-50A protein (SEQ ID NO: 144). As component B, the VLP uses the I53-50B protein. It is known that I53-50A and I53-50B spontaneously assemble in 3:5 ratio to form a VLP having icosahedral (I53) symmetry, as shown in **FIG. 1A and B**.

[0206] The expression level of hMPV F proteins fused to CompA were determined. Human codon-optimized polynucleotide sequences were made by gene synthesis and cloned into expression vectors. Each expression vector was individually expressed by transient transfection in Expi293 cells. Supernatants were collected four days after transfection.

[0207] To determine if the recombinant expressed fusion proteins were secreted into the supernatant, a Western blot using an anti-His6 monoclonal antibody was performed. Conditioned media were diluted a minimum of 10-fold in 2X Laemmli loading buffer (Bio-Rad) with 5% (v/v) β -mercaptoethanol and heated to 95°C for 10 minutes and 10 μ L run on a gel. Reference samples of recombinant His-tagged human serum albumin (HSA) were prepared by an initial dilution to 0.01 mg/mL, followed by a 2-fold dilution series to obtain a standard curve from 6.25 to 100 ng in a 10 μ L loading volume in 2X Laemmli loading buffer. Samples were loaded onto a NuPAGE 4-12% Bis-Tris protein gel and run for 45 minutes at 150V. Proteins were transferred onto an Immuno-Blot PDVF membrane (Bio-Rad). Following transfer, the blot was blocked with 3% BSA (w/v) in TBST for 1 hour at room temperature with shaking. An anti-His, HRP-conjugated antibody (R&D Systems MAB050H) was diluted 1:8,000 in 3% BSA (w/v) in TBST and incubated with the membrane for 1 hour at room temperature. The membrane was then washed three times for five minutes using TBST, then His-tagged protein was detected using a luminol chemiluminescent substrate (VisiGlo, VWR) and captured using a 10-20 second exposure time on a UVP Chemstudio (Analytik Jena).

[0208] Table 4 shows the expression levels of hMPV F protein fused to CompA. Expression levels were assessed by visual inspection of Western blots and designated as no expression (X) or low expression (*) to high expression (****). The hMPV008, hMPV009, hMPV024, hMPV026, hMPV027, hMPV033, and hMPV034 CompA fusion proteins showed the highest expression levels in this system.

Table 4. hMPV F-CompA Fusion Proteins Tested for Expression in Expi293 cells.

Construct Name	Mutations	hMPV strain (A or B)	CompA: Ala or Cys	C-term Truncation	Expression Level
hMPV001	A185P, Q100R, S101R	A	Ala	481	*
hMPV002	A185P, Q100R, S101R	A	Ala	472	**
hMPV003	A185P, Q100R, S101R	A	Ala	489	*
hMPV004	Q100R, S101R	A	Ala	472	*
hMPV005	A185P, T127C, N153C, Q100R, S101R	A	Ala	472	**
hMPV006	V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G	B	Ala	472	**
hMPV007	V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G	B	Ala	488	**
hMPV008	A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G	B	Ala	472	****
hMPV009	A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G	B	Ala	488	****
hMPV010	A63C, A140C, A147C, K188C, G106 deleted,	A	Ala	472	X

Construct Name	Mutations	hMPV strain (A or B)	CompA: Ala or Cys	C-term Truncation	Expression Level
	N97G, P98G, R99G, Q100G, S101G, R102G				
hMPV011	A63C, A140C, A147C, K188C, G106 deleted, N97G, P98G, R99G, Q100G, S101G, R102G	A	Ala	488	X
hMPV012	A113C, A120C, A339C, Q426C, T160F, I177L, Q100K, S101A	B	Ala	472	X
hMPV013	A113C, A120C, A339C, Q426C, T160F, I177L, Q100K, S101A	B	Ala	488	X
hMPV014	V84C, A140C, A147C, A249C, Q100R, S101R	B	Ala	472	**
hMPV015	A63C, A140C, A147C, K188C, K450C, S470C, Q100R, S101R	B	Ala	472	***
hMPV016	A63C, A140C, A147C, K188C, G106 deleted, Q100R, S101R	A	Ala	472	X
hMPV017	A113C, A120C, A339C, Q426C, T160F, I177L, Q100R, S101R	B	Ala	472	X
hMPV018	A185P, A113C, A339C, Q100R, S101R	A	Ala	472	**
hMPV019	A185P, T160F, I177L, Q100R, S101R	A	Ala	472	*
hMPV020	A185P, A113C, A339C, T160F, I177L, Q100R, S101R	A	Ala	472	*

Construct Name	Mutations	hMPV strain (A or B)	CompA: Ala or Cys	C-term Truncation	Expression Level
hMPV021	A63C, K188C, N97G, P98G, R99G, Q100G, S101G, R102G	B	Ala	472	***
hMPV022	A63C, K188C, K450A, S470A, N97G, P98G, R99G, Q100G, S101G, R102G	B	Ala	472	***
hMPV023	A63C, A140C, A147C, K188C, G294E, N97G, P98G, R99G, Q100G, S101G, R102G	A	Ala	472	**
hMPV024	A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G, G294E	A	Ala	472	****
hMPV025	A63C, K188C, N97G, P98G, R99G, Q100G, S101G, R102G, G294E	A	Ala	472	**
hMPV026	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, Q100R, S101R	A	Ala	472	****
hMPV027	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, Q100R, S101R	A	Ala	490	****
hMPV028	A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G	B	Cys	472	*

Construct Name	Mutations	hMPV strain (A or B)	CompA: Ala or Cys	C-term Truncation	Expression Level
hMPV029	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, Q100R, S101R	A	Cys	472	*
hMPV030	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, Q100R, S101R	A	Cys	490	*
hMPV031	V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G	B	Cys	472	*
hMPV033	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, N97G, P98G, R99G, Q100G, H368N, S101G, R102G	A	Ala	472	****
hMPV034	T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, N97G, P98G, R99G, H368N, Q100G, S101G, R102G	A	Ala	490	****

Example 2

[0209] In this example, conditioned media containing the hMPV F-CompA fusion protein were characterized using antibody binding activity to determine the conformation of the hMPV F protein. The fusion proteins hMPV005, hMPV008, hMPV021, hMPV024, hMPV026, hMPV027, hMPV027C, and hMPV033 were evaluated (Table 5). Constructs hMPV026 and hMPV027 contain a furin cleavage site that was not cleaved in the Expi293 transient transfections. Upon co-transfection of human furin, the expressed protein was properly cleaved and termed hMPV026C or hMPV027C to represent the cleaved form.

[0210] To characterize the conformation of the recombinant expressed hMPV F protein fused to CompA, an ELISA was performed using 15 monoclonal antibodies that bind to specific forms of the hMPV F protein ectodomain.

[0211] Wells of a 96 well plate were coated with 200 ng of each antibody, or 50 ng for MPE8, in 100 μ L 50 mM sodium carbonate-bicarbonate buffer, pH 9.6, at 4°C overnight. Plates were washed 3X with 300 μ L PBS with 0.05% Tween 20. After blocking with 150 μ L PBS with 1% BSA for 1 hour at room temperature, the plates were emptied by tapping onto an absorbent pad. Conditioned media supernatants from the Expi293 transfections of each construct were diluted based on semi-quantitative Western blots such that antigen concentrations would bracket the expected EC₅₀ of the antigen (from Mas data). Three dilutions for each construct were plated at 100 μ L per well for each antibody and the plates were incubated for 1 hour at room temperature. Plates were washed 6X with 300 μ L PBS with 0.05% Tween 20. An anti-His monoclonal antibody (R&D Systems MAB050H) was diluted 1:15,000 and 100 μ L was added to each well and the plate incubated for 1 hour at room temperature. After washing the plates 6X with 300 μ L PBS with 0.05% Tween 20, 100 μ L TMB substrate was added and the plate was developed in the dark for 10 minutes. The reaction was stopped by addition of 100 μ L 0.6 N H₂SO₄ and absorbance was measured at 450 nm on a plate reader.

[0212] The postfusion specific antibodies bound hMPV021 suggesting this construct has a postfusion conformation. The other constructs did not bind the postfusion specific Abs and did bind MPE8 along with multiple Abs that recognize both prefusion and postfusion conformation. This data suggest the fusion proteins have prefusion conformation characteristics.

Example 3

Binding activity seen with purified hMPV F-CompA fusion proteins. **FIGs. 3A-3C** show binding with known concentrations of purified component A (CompA) fusions—that is, hMPV F protein ectodomains N-terminally fused to I53-50A (SEQ ID NO: 144) or I53-50A Δ Cys (SEQ ID NO: 145). MF14, MPE8, and MF16 recognize antigenic sites II, III, and IV respectively and these antigenic sites are the primary sites recognized by neutralizing antibodies. In addition, MPE8 recognizes preferentially the prefusion form of hMPV F protein. Wells of a 96 well plate were coated with 50 ng of MPE8 and 100 ng of MF14 and MF16 in 100 μ L 50 mM sodium carbonate-bicarbonate buffer, pH 9.6, at 4°C overnight. Plates were washed 3X with 300 μ L PBS with 0.05% Tween 20. After blocking with 150 μ L PBS with 1% BSA for 1 hour at room temperature, the plates were emptied by tapping onto an absorbent

pad. Purified samples of each construct were diluted to 40 µg/mL (hMPV008, 021, 026, and 027) or 4 µg/mL (hMPV026C and 033), followed by a 3-fold or 4-fold dilution series respectively in PBS with 1% BSA and plated at 100 µL per well and the plates were incubated for 1 hour at room temperature. Plates were washed 6X with 300 µl PBS with 0.05% Tween 20. An anti-His monoclonal antibody (R&D Systems MAB050H) was diluted 1:15,000 and 100 µL was added to each well and the plate incubated for 1 hour at room temperature. After washing the plates 6X with 300µl PBS with 0.05% Tween 20, 100 µl TMB substrate was added and the plate was developed in the dark for 10 minutes. The reaction was stopped by addition of 100µl 0.6 N H₂SO₄ and absorbance was measured at 450 nm on a plate reader. Binding to MF14 (site II, **FIG. 3A**) and MF16 (site IV, **FIG. 3B**) are similar but binding with MPE8 (site III, **FIG. 3C**) has a higher EC₅₀ than with hMPV008 and hMPV021 (**Table 6**). This suggests that site III, a neutralizing antibody recognition site, is more conserved in hMPV026, hMPV027, and hMPV033 and these constructs more preferentially adopt the prefusion conformation than hMPV008 and hMPV021.

Table 6. Neutralizing Antibody Binding EC₅₀ Values

Construct:	EC ₅₀		
	MPE8	MF14	MF16
hMPV008	8.19E+00	1.78E-01	1.99E-01
hMPV021	1.28E+01	1.66E-01	1.86E-01
hMPV026	7.70E-02	9.57E-02	1.01E-01
hMPV026C	1.24E-01	1.36E-01	1.40E-01
hMPV027	7.96E-02	9.37E-02	9.65E-02
hMPV033	9.68E-02	1.08E-01	1.17E-01

Example 4

[0213] In this example, assembled hMPV008 VLPs or hMPV008 CompA alone were injected intramuscular in both hind legs of female Balb/c mice on day 0 and day 21. The quantity and formulation of each group is listed in Table 7. Blood draws were collected on days 0, 24 and 35 and processed into serum. Day 0 serum samples were pooled by group. All serum samples were evaluated in a hMPV neutralization antibody titer assay (**FIG. 4**). hMPV008 formulations with adjuvants Alhydrogel (Alum) or Addavax (squalene oil-in-water emulsion) induced measurable neutralizing titers at all doses at day 24 and 35, and were comparable to titers of healthy adult serum (**FIG. 4**). These results show that hMPV008 VLPs induce robust neutralizing titers comparable to healthy human control sera.

Table 7. Quantity and Formulations of Injected VLPs

Antigen	Dose	Formulation
hMPV008 VLP	5 µg	Addavax
hMPV008 VLP	5 µg	Alhydrogel
hMPV008 VLP	0.25 µg	Alhydrogel
hMPV008 CompA	4.08 µg	Alhydrogel
hMPV008 CompA	0.2 µg	Alhydrogel

Table 5. Antibody Binding to Illustrative CompA Fusion Proteins

Ab	Ag site	Pre/Post Preference	Neut A/B	hMPV005	hMPV008	hMPV021	hMPV024	hMPV026‡	hMPV027‡	hMPV027C	hMPV033
MF1	6HB	post	No	low	NB	very high	low	NB	NB	NB	NB
MF2	6HB	post	No	low	NB	very high	low	NB	NB	NB	NB
MF3	6HB	post	No	low	NB	medium	NB	NB	NB	NB	NB
MF9	Site II	pre/post (B preferential)	A/B	very high	very high	very high	very high	very high	very high	very high	very high
MF12	Site II	pre/post	A/B	very high	very high	very high	very high	very high	very high	very high	very high
MF14	Site II	pre/post	A/B	very high	very high	very high	very high	very high	very high	very high	very high
MF15	Site II	pre/post	A/B	very high	very high	very high	very high	very high	very high	very high	very high
MF11	Site IV	post preferential	No	medium	low	very high	high	NB	NB	NB	NB
MF16	Site IV	pre/post	A/B	very high	very high	very high	high	very high	very high	very high	very high
MF20	Site IV	pre/post (B preferential)	A/B	very high	medium	very high	medium	low	low	low	low
MF17	Site I	post preferential	B	high	NB	high	low	low	low	low	low
MF18	Site I	post preferential	B	very high	low	very high	medium	low	low	low	low
MF19	Site I	post preferential	B	very high	medium	very high	medium	low	low	low	medium
MF10	Site 0	Pre (A preferential)	A	very high	NB	NB	low	very high	very high	very high	very high
MPE8	Site III	pre	A/B	high	high	high	high	high	high	high	high

* A very high response indicates OD450 > 2.0, a high response indicates an OD450 > 1.0 but less than 2.0, medium response indicates OD450 > 0.5 but < 1.0 and low response indicates OD450 > 0.1 but < 0.5 and NB (no binding) is < 0.1. ‡ Construct retains native furin cleavage site.

Example 5

[0214] A study was undertaken in naïve mice to explore the immunogenicity hMPV F protein mutants displayed on a 2-component virus-like particle (VLP). VLPs containing one of four different hMPV F protein mutants, hMPV008, hMPV024, hMPV026C, and hMPV033 or a soluble protein corresponding to hMPV026C were tested. Each group consisted of 8 female Balb/c mice. Mice were immunized on day 0 and day 21. All test articles were formulated with Addavax, an oil in water adjuvant. Two dose levels (1 µg and 0.25 µg) or an equivalent antigen content of soluble protein were administered (Table 8). Neutralizing antibody titers against hMPV/A and hMPV/B were determined from day 35 serum (FIG. 5A and 5B, respectively). The results demonstrated robust neutralizing titers were induced by all VLPs against both strains of hMPV. The soluble protein also induced titers against both hMPV strains at a lower level than the VLPs.

Table 8: Quantity and Formulations of Injected VLPs

Antigen	Dose	Formulation
hMPV033 VLP	1 µg	Addavax
hMPV008 VLP	1 µg	Addavax
hMPV024 VLP	1 µg	Addavax
hMPV026C VLP	1 µg	Addavax
hMPV033 VLP	0.25 µg	Addavax
hMPV008 VLP	0.25 µg	Addavax
hMPV024 VLP	0.25 µg	Addavax
hMPV026C VLP	0.25 µg	Addavax
Soluble hMPV026C	0.64 µg	Addavax
Soluble hMPV026C	0.16 µg	Addavax
No treatment	N/A	N/A

Example 6

[0215] A study was undertaken in naïve mice to explore the immunogenicity of hMPV008 VLPs and the corresponding soluble protein, CompA-hMPV008. hMPV008 VLPs and CompA-hMPV008 were formulated in aqueous buffer, a squalene-based adjuvant (SE), or Alhydrogel at two dose levels (1 µg and 0.1 µg VLP and the equivalent antigen dose for CompA-hMPV008). In addition, one group with hMPV033 VLP (0.1 µg) formulated with SE was included (Table 9). Each group consisted of 8 female Balb/c mice. Mice were immunized on day 0 and day 21. Neutralizing antibody titers against hMPV/A and hMPV/B were

determined from day 35 serum. Results demonstrated that hMPV008 VLPs formulated with SE resulted in higher titers than Alhydrogel or aqueous formulations (Figs. 6A and 6B). VLPs induced higher neutralizing titers than the soluble protein CompA-hMPV008 with all formulations (Figs. 6A and 6B).

Table 9: Quantity and Formulations of Injected VLPs

Antigen	Dose	Formulation
hMPV008 VLP	1 µg	SE
hMPV008 VLP	1 µg	Alhydrogel
hMPV008 VLP	1 µg	Aqueous
hMPV008 VLP	0.1 µg	SE
hMPV008 VLP	0.1 µg	Alhydrogel
hMPV008 VLP	0.1 µg	Aqueous
CompA-hMPV008	0.81 µg	SE
CompA-hMPV008	0.81 µg	Alhydrogel
CompA-hMPV008	0.81 µg	Aqueous
CompA-hMPV008	0.081 µg	SE
CompA-hMPV008	0.081 µg	Alhydrogel
CompA-hMPV008	0.081 µg	Aqueous
hMPV033	0.1 µg	SE

Example 7

[0216] A study was undertaken in naïve mice to explore the immunogenicity VLPs displaying an hMPV F protein mutant, hMPV008. Each group consisted of 8 female Balb/c mice. Mice were immunized on day 0 and day 21 with hMPV008 formulated with Addavax, an oil in water adjuvant. Two dose levels (1 ug and 0.2 ug) of VLP were administered as indicated in Table 10. Neutralizing antibody titers were determined for hMPV/A and hMPV/B from serum samples collected on day 35. Results demonstrated that robust hMPV/A and hMPV/B neutralizing titers were induced by hMPV008 with both dose levels (FIGs. 7A and 7B).

Table 10: Quantity and Formulations of Injected VLPs

Antigen	Dose	Formulation
hMPV008	1 µg	Addavax
hMPV008	0.2 µg	Addavax

Example 8

[0217] A study was undertaken in naïve mice to explore the immunogenicity of hMPV033. Each group consisted of 8 female Balb/c mice. Mice were immunized on day 0 and 21 with hMPV033 formulated with Addavax, an oil in water adjuvant, at 4 dose levels (4, 2, 1, and 0.5 µg) (Table 11). Neutralizing antibody titers were determined for hMPV/A and hMPV/B from serum samples collected on day 35. Results demonstrated that robust hMPV/A and hMPV/B neutralizing titers were induced by hMPV033 with all dose levels (FIGs. 8A and 8B, respectively).

Table 11: Quantity and Formulations of Injected VLPs

Antigen	Dose	Formulation
hMPV033	4 µg	Addavax
hMPV033	2 µg	Addavax
hMPV033	1 µg	Addavax
hMPV033	0.5 µg	Addavax

Example 9

[0218] A study was undertaken in naïve cotton rats to assess and evaluate the ability of hMPV033 to protect against hMPV infection in cotton rats. hMPV033 was administered on day 0 and 21 with a dose level of 1 µg formulated with Addavax (Table 12). Each group consisted of 8 female cotton rats. Neutralizing titers against hMPV/A were determined from day 35 serum samples (FIG. 9A). Groups were challenged with 10⁵ pfu hMPV on day 35 (Table 12) and sacrificed on day 40. Viral titers were determined from lung or nasal turbinates in a plaque assay. Results demonstrated that hMPV033 induced robust neutralizing titers (FIG. 9A). Plaque assays on lung and nasal turbinate tissue homogenates demonstrated that high viral titers were detected in lung and nasal turbinates following challenge of control animals. In contrast, viral titers in vaccinated cotton rats were below the lower limit of detection for lung and nasal turbinate samples. (FIGs. 9B and 9C). Formalin inactivated hMPV vaccination did not protect against subsequent challenge (FIGs. 9B-9C).

Table 12: Quantity and Formulations of Injected VLPs

Antigen	Dose	Formulation	hMPV challenge
PBS	N/A	N/A	No
PBS	N/A	N/A	Yes
hMPV033	1 µg	Addavax	Yes

Antigen	Dose	Formulation	hMPV challenge
Formalin inactivated hMPV		N/A	Yes

* * * *

[0219] While the invention has been described in connection with proposed specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

CLAIMS

What is claimed is:

1. A virus-like particle (VLP), comprising a first component and optionally a second component,
wherein the first component is a fusion protein, comprising a human metapneumovirus (hMPV) F protein ectodomain or antigenic variant thereof, and a first multimerization domain; and
wherein the second component, if present, is a protein comprising a second multimerization domain.
2. The VLP of claim 1, wherein the first multimerization domain is a trimerization domain.
3. The VLP of claim 1 or claim 2, wherein the first multimerization domain is selected from SEQ ID NOS: 1, 4, 5, 7, 9, 18, 19, 21, 24, 25, 26, 29, 30, 31, 34, 36, 37, 39, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 144, 145, or functional variants thereof.
4. The VLP of any one of claims 1 to 3, wherein the first multimerization domain is I53-50A (SEQ ID NO: 144) or a functional variant or variant thereof.
5. The VLP of any one of claims 1 to 3, wherein the first multimerization domain shares at least 70%, at least 80%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% identity to

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1 MEELFKKKHI VAVLRANSVE EAIEKAVAVF AGGVHLIEIT
41 FTVPDADTVI KALSVLKEKG AIIGAGTVTS VEQCRKAVES
81 GAEFIVSPHL DEEISQFCKE KGVFYMPGVM TPTELVKAMK
121 LGHTILKLFP GEVVGPFQFVK AMKGPFPNVK FVPTGGVNLD
161 NVCEWFKAGV LAVGVGSALV KGTPDEVREK AKAFVEKIRG
201 CTE (SEQ ID NO: 144)

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or

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1 MEELFKKKHI VAVLRANSVE EAIEKAVAVF AGGVHLIEIT
41 FTVPDADTVI KALSVLKEKG AIIGAGTVTS VEQARKAVES
81 GAEFIVSPHL DEEISQFAKE KGVFYMPGVM TPTELVKAMK
121 LGHTILKLFP GEVVGPFQFVK AMKGPFPNVK FVPTGGVNLD
161 NVAEWFKAGV LAVGVGSALV KGTPDEVREK AKAFVEKIRG
201 ATELE (SEQ ID NO: 145).

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6. The VLP of claim 5, wherein the first multimerization domain comprises:
the amino acid substitutions C74A and C98A;
the amino acid substitutions C163A and C201A; or
the amino acid substitutions C74A, C98A, C163A, and C201A relative to SEQ ID NO: 144.
7. The VLP of any one of claims 1 to 3, wherein the first multimerization domain has a polypeptide sequence identical to SEQ ID NO: 144.
8. The VLP of any one of claims 1 to 3, wherein the first multimerization domain has a polypeptide sequence identical to SEQ ID NO: 145.
9. The VLP of any one of claims 1 to 9, wherein the first multimerization domain is a VLP-forming domain.
10. The VLP of any one of claims 1 to 9, wherein the first multimerization domain is adapted to drive assembly of an icosahedral particle or tetrahedral particle, optionally with a second multimerization domain.
11. The VLP of any one of claims 1 to 10, wherein second multimerization domain is selected from SEQ ID NOs: 2, 3, 6, 8, 10, 11, 12, 13, 14, 15, 16, 17, 20, 22, 23, 27, 28, 32, 33, 35, 38, 40, and 41 or functional variants and fragments thereof.
12. The VLP of any one of claims 1 to 11, wherein the second multimerization domain is I53-50B (SEQ ID NO: 8), I53-50B.4PosT1 (SEQ ID NO: 34), or a functional variant thereof.
13. The VLP of any one of claims 1 to 12, wherein the (hMPV) F protein ectodomain or antigenic variant thereof and the first multimerization domain are joined by a linker sequence.
14. The VLP of claim 13, wherein the linker sequence comprises a foldon, wherein the foldon sequence is EKAAKAEEAARK (SEQ ID NO: 125).
15. The VLP of claim 13 or 14, wherein the linker sequence comprises GSGGSGSGSGGS (SEQ ID NO: 126).

16. The VLP of any one of claims 1 to 15, wherein the hMPV F protein ectodomain contains one or more substitutions or deletions selected from A185P, Q100R, S101R, T127C, N153C, V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, G106 deletion, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, I177L, K450A, S470A, G294E, T365C, V463C, L219K, H368N, and/or V231I.

17. The VLP of any one of claims 1 to 15, wherein the hMPV F protein ectodomain contains one or more substitutions or deletions selected from V84C, A140C, A147C, N97G, P98G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, R99G, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, Q100R, S101R, G106 deletion, S101R, A185P, I177L, and/or G294E.

18. The VLP of any one of claims 1 to 15, wherein the hMPV F protein ectodomain contains two or more substitutions or deletions selected from V84C, A140C, A147C, N97G, P98G, Q100G, S101G, R102G, A63C, K188C, K450C, S470C, R99G, A113C, A120C, A339C, Q426C, T160F, Q100K, S101A, Q100R, S101R, G106 deletion, S101R, A185P, I177L, and/or G294E.

19. The VLP of any one of claims 1 to 15, wherein the hMPV F protein ectodomain contains one or more substitutions selected from A185P, Q100R, S101R, T127C, N153C, T365C, V463C, L219K, V231I, G294E, N153C, N97G, P98G, R99G, Q100G, S101G, H368N, and/or R102G.

20. The VLP of any one of claims 1 to 15, wherein the hMPV F protein ectodomain contains two or more of A185P, Q100R, S101R, T127C, N153C, T365C, V463C, L219K, V231I, G294E, N153C, N97G, P98G, R99G, Q100G, S101G, H368N, and/or R102G.

21. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises:

- a. the substitutions Q100R and S101R;
- b. the substitutions A185P, Q100R, and S101R;
- c. the substitutions A185P, T127C, N153C, Q100R, and S101R
- d. the substitutions V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, and R102G;

- e. the substitutions A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, and R102G;
- f. the substitutions and deletion A63C, A140C, A147C, K188C, G106 deletion, N97G, P98G, R99G, Q100G, S101G, and R102G;
- g. the substitutions A113C, A120C, A339C, Q426C, T160F, I177L, Q100K, and S101A;
- h. the substitutions V84C, A140C, A147C, A249C, Q100R, and S101R;
- i. the substitutions A63C, A140C, A147C, K188C, K450C, S470C, Q100R, and S101R;
- j. the substitutions and deletion A63C, A140C, A147C, K188C, G106 deletion, Q100R, and S101R;
- k. substitutions A113C, A120C, A339C, Q426C, T160F, I177L, Q100R, and S101R;
- l. substitutions A185P, A113C, A339C, Q100R, and S101R;
- m. substitutions A185P, T160F, I177L, Q100R, and S101R;
- n. substitutions A185P, A113C, A339C, T160F, I177L, Q100R, and S101R;
- o. substitutions A63C, K188C, N97G, P98G, R99G, Q100G, S101G, and R102G;
- p. substitutions A63C, K188C, K450A, S470A, N97G, P98G, R99G, Q100G, S101G, and R102G;
- q. substitutions A63C, A140C, A147C, K188C, G294E, N97G, P98G, R99G, Q100G, S101G, and R102G;
- r. substitutions A63C, A140C, A147C, K188C, K450C, S470C, N97G, P98G, R99G, Q100G, S101G, R102G, and G294E;
- s. substitutions A63C, K188C, N97G, P98G, R99G, Q100G, S101G, R102G, and G294E;
- t. substitutions T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, H368N, Q100R, and S101R;
- u. substitutions V84C, A140C, A147C, A249C, N97G, P98G, R99G, Q100G, S101G, and R102G; or
- v. substitutions T127C, N153C, T365C, V463C, A185P, L219K, V231I, G294E, N97G, P98G, R99G, Q100G, S101G, H368N, and R102G.

22. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 58.

23. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 59.

24. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 60.

25. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 61.

26. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 62.

27. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 63.

28. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 64.

29. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 65.

30. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 66.

31. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 67.

32. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 68.

33. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 69.

34. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 70.

35. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 71.

36. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 72.

37. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 73.

38. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 74.

39. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 75.

40. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 76.

41. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 77.

42. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 78.

43. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 79.

44. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 80.

45. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 81.

46. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 82.

47. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 83.

48. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 84.

49. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 85.

50. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 86.

51. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 87.

52. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 88.

53. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 89.

54. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain comprises a sequence that shares at least 95% or at least 99% identity to SEQ ID NO: 90.

55. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 58, 60, 61, 77, 78, 86, 87, 88, or 89.

56. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 59, 62, 63, 64, 71, 76, 81, or 83.

57. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 72, 79, or 80.

58. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 65, 66, 82, 84, 85, 90, or 91.

59. The VLP of any one of claims 1 to 20, wherein the hMPV F protein ectodomain shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 65, 79, or 80.

60. The VLP of any one of claims 1 to 59, wherein the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 92-124.

61. The VLP of any one of claims 1 to 59, wherein the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 97-116, 119, or 122.

62. The VLP of any one of claims 1 to 59, wherein the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 92-94, 96, 117, 118, 120, 121, 123, or 124.

63. The VLP of any one of claims 1 to 59, wherein the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 99, 112, 117, or 118.

64. The VLP of any one of claims 1 to 63, wherein the VLP binds one or more hMPV F protein antibodies selected from MF1, MF2, MF3, MF9, MF12, MF14, MF15, MF11, MF16, MF20, MF17, MF18, MF19, MF10, or MPE8.

65. The VLP of any one of claims 1 to 63, wherein the VLP binds two or more antibodies selected from MF1, MF2, MF3, MF9, MF12, MF14, MF15, MF11, MF16, MF20, MF17, MF18, MF19, MF10, MPE8.

66. The VLP of any one of claims 1 to 63, wherein the VLP binds three or more antibodies selected from MF1, MF2, MF3, MF9, MF12, MF14, MF15, MF11, MF16, MF20, MF17, MF18, MF19, MF10, MPE8.

67. The VLP of any one of claims 1 to 63, wherein the hMPV F protein ectodomain binds an antibody that preferentially binds the prefusion form of the hMPV F protein ectodomain.
68. The VLP of any one of claims 64 to 67, wherein the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 42, 45, 58, 61, 63, or 64.
69. The VLP of any one of claims 1 to 63, wherein the hMPV F protein ectodomain has no or low binding to an antibody that preferentially binds the postfusion form of the hMPV F protein ectodomain.
70. The VLP of claim 69, wherein the VLP sequence shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity to SEQ ID NOs: 99, 115, 117, or 118.
71. The VLP of claim 1, wherein the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity with SEQ ID NO: 99.
72. The VLP of claim 1, wherein the first component is a single chain.
73. The VLP of claim 37, wherein the single chain shares at least 85%, at least 90%, at least 95%, at least 99% or 100% identity with SEQ ID NO: 123.
74. The VLP of claim 1, wherein the first component shares at least 85%, at least 90%, at least 95%, at least 99%, or 100% identity with SEQ ID NO: 117.
75. A polynucleotide encoding the VLP of any one of claims 1 to 74.
76. A host cell, comprising the polynucleotide of claim 75.
77. A method of manufacturing a vaccine, comprising culturing the host cell of claim 76 in a culture medium so that the host cell secretes the antigen into the culture media; optionally purifying the antigen from the culture media; mixing the antigen with a second component, wherein the second component multimerizes with the antigen to form a virus-like particle; and optionally purifying the virus-like particle.

78. A vaccine, comprising the VLP of any one of claims 1 to 74, wherein the vaccine optionally comprises one or more pharmaceutically acceptable diluents, adjuvants, or excipients.
79. The vaccine of claim 78, wherein the vaccine is a stable emulsion.
80. The vaccine of claim 78 or claim 79, wherein the vaccine comprises one or more adjuvants.
81. The vaccine of claim 80, wherein the one or more adjuvants is squalene, alum, SLA, GLA, R848, IMQ, 3M-052, CpG, saponin (QS21), or combinations thereof.
82. The vaccine of claim 80, wherein the adjuvant is alum.
83. The vaccine of claim 80, wherein the adjuvant is a squalene-based emulsion.
84. The vaccine of claim 83, wherein the squalene-based emulsion is MF59.
85. A method of immunizing a subject against infection by human metapneumovirus, the method comprising administering the vaccine of any one of claims 78 to 84.
86. The method of claim 85, wherein the subject is simultaneously immunized against infection by respiratory syncytial virus (RSV).
87. The method of claim 85, wherein the vaccine is administered by subcutaneous injection.
88. The method of claim 85, wherein the vaccine is administered by intramuscular injection.
89. The method of claim 85, wherein the vaccine is administered by intradermal injection.
90. The method of claim 85, wherein the vaccine is administered intranasally.
91. A pre-filled syringe comprising the vaccine of any one of claims 78 to 84.

92. A kit comprising the vaccine of any one of claims 78 to 84 or the pre-filled syringe of claim 91.

93. The method of claim 77, wherein the host cell is transfected with a polynucleotide encoding furin or a functional variant thereof.