78:12557–12565) described formation of VLPs in transfected human cells that only required co-expression of the M and N viral proteins, whereas Siu et al. (Journal of Virology (2008)82:11318–11330) showed that both E and N proteins must be co-expressed with M protein for the efficient production and release of SARS-CoV VLPs in transfected mammalian cells.

[0021] WO2012/083445 discloses the production of SARS CoV S protein in plants, wherein the transmembrane domain and the cytosolic tail domain (TM/CT) of the S protein were replaced with TM/CT from an influenza HA protein.

[0022] A few groups have proposed immunization with SARS-CoV VLPs as an effective vaccine strategy. VLPs produced in insect cells or chimeric MHV/SARS-CoV VLPs produced in mammalian cells were used in these studies (Lokugamage et al. Vaccine 2008 Feb 6;26(6):797-808, Lu et al. 2007 Immunology 122496-5024).

[0023] However, effective scale-up and manufacture of SARS-CoV-2 VLPs at the quantity required to meet the need of widespread vaccination of the global population, requires efficient viral structural protein and VLP production.

SUMMARY

[0024] The present disclosure relates to modified viral structural proteins. The present disclosure also relates to virus-like particles (VLPs) comprising modified viral structural protein and methods of producing the VLPs in a host or host cells. More specifically, the disclosure relates to modified coronavirus S proteins. The present disclosure also relates to virus-like particles (VLPs) comprising modified S proteins and methods of producing the VLPs in a host or host cells.

[0025] In one aspect it is provided a modified coronavirus S-protein comprising, in series,

- an ectodomain derived from a coronavirus S-protein,

- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:

- a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein, and

- a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein.

[0026] The modified S-protein as described herein may form trimers. Accordingly it is also provided a trimer comprising modified coronavirus S-protein as described herewith.

[0026a] In another aspect, the present disclosure refers to a trimer comprising the modified S-protein as disclosed herein.

[0027] In a further example, a virus like particle (VLP) comprising the modified S-protein or trimers comprising the modified S-protein as described above is provided. In another aspect, the present disclosure refers to a virus like particle (VLP) comprising the modified S-protein as disclosed herein or the trimer as disclosed herein. Accordingly, the VLP comprises a modified coronavirus S-protein or trimer that comprise the modified S-protein, the modified S-protein comprising

- an ectodomain derived from a coronavirus S-protein,

- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:

  - a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and

  - a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein.

[0028] The VLP may further comprise plant lipids.

[0029] The TM may be directly fused to the CT. The TM may be derived from the coronavirus S-protein TM and the CT may be derived from the influenza HA protein CT. Furthermore, the TM may be a chimeric TM comprising a N terminal sequence derived from the coronavirus S-protein TM and a C terminal sequence derived from the influenza HA protein TM. The chimeric TM may comprise a N terminal sequence derived from the coronavirus S-protein TM comprising at least 20 amino acids corresponding to amino acids 1-20 of SEQ ID NO: 18 or SEQ ID NO: 169, or at least 21 amino acids corresponding to amino acids 1-21 of SEQ ID NO: 118 or 164, or at least 22 amino acids corresponding to amino acids 1-22 of SEQ ID NO: 123 and one or more than one amino acid from the C-terminal end of the influenza HA protein TM. The one or more than one

amino acid from the C-terminal end of the influenza HA protein TM may be selected from AGL or conserved substitution of AGL, MAGL or conserved substitution of MAGL. The chimeric TM may comprise amino acids corresponding to amino acids of 1-20 of SEQ ID NO: 18.

[0030] The CT may be chimeric CT comprising a N terminal sequence derived from the coronavirus S-protein CT and a C terminal sequence derived from the influenza HA protein CT. The chimeric CT may comprise a C terminal sequence derived from influenza HA protein CT comprising at least 11 amino acids corresponding to amino acids 27-37 of SEQ ID NO: 18, 126, 127, 128, 129, 130 or 131 and one or more than one amino acid from the N-terminal end of the coronavirus S-protein CT. The one or more than one amino acid from the N-terminal end of the coronavirus S-protein CT may be selected from C or a conserved substitution of C, CC or a conserved substitution of CC, or CCM or a or a conserved substitution of CCM. The chimeric CT may comprise amino acids corresponding to amino acids of 27-37 of SEQ ID NO: 18, 126, 128, 129, 130 or 131; or amino acids 27-36 of SEQ ID NO: 127. In one example the chimeric TMCT may comprise a chimeric TM comprising amino acids corresponding to amino acids 1-20 of SEQ ID NO: 18 or SEQ ID NO: 169, or to amino acids 1-21 of SEQ ID NO: 118 or SEQ ID NO: 164, or amino acids 1-22 of SEQ ID NO: 123, a chimeric CT comprising amino acids corresponding to amino acids 27-37 of SEQ ID NO: 18, 126, 127, 128, 129, 130 or 131, or a combination thereof.

[0031] The CT or portion of the CT may comprise from 80% to 100% identity with the sequence of SEQ ID NO: 15, or with amino acids 35-50 of SEQ ID NO 6, 8, 7, 9, 10, 12, 13 or 14, or with amino acids 34-49 of SEQ ID NO 11, or with amino acids 553-568 of SEQ ID NO:3 or with amino acids 22-37 of SEQ ID NO:18, or with amino acids 21-40 of SEQ ID NO: 19, or with amino acids 21-39 of SEQ ID NO: 37, or with amino acids 25-36 of SEQ ID NO: 38 or with amino acids 24-34 of SEQ ID NO: 39, or amino acids 22-37 of SEQ ID NO: 126, 128, 129, 130 or 131; or amino acids 22-36 of SEQ ID NO: 127. The TM or portion of the TM may comprises from 80% to 100% identity with the sequence of SEQ ID NO: 132 or 133.

[0038] In a further example, the modified S-protein may comprise one or more than one amino acid substitution when compared to a wild-type coronavirus amino acid sequence. The one or more than one substitution may maintain the S-protein in a pre-fusion state.

[0039] The one or more than one amino acid substitution may comprise i) substitutions that restricts the processing at a cleavage site between S1 and S2 subunit, ii) substitution of one or more than one amino acid to one or more than one proline, or iii) substitutions that restrict the processing at the cleavage site between the S1 and the S2 subunit and substitution of one or more than one amino acid to one or more than one proline.

[0040] The one or more than one substitution may maintain the S-protein in a pre-fusion state or produces are higher yield of the modified S-protein when expressed in a host or host cell, when compared to the yield of a corresponding S-protein without the one or more than one substitutions expressed in the host or host cell.

[0041] The one or more than one amino acid substitution may correspond to amino acids at positions 667, 668, 670, 802, 923, 927, 971, 972 or a combination thereof, when compared to reference amino acid sequence of SEQ ID NO: 2.

[0042] In one example, the one or more than one amino acid substitution correspond to amino acids at positions 971 and 972, when compared to reference amino acid sequence of SEQ ID NO: 2. In another example, the one or more than one amino acid substitution correspond to amino acids at positions 802, 927, 971 and 972, when compared to reference amino acid sequence of SEQ ID NO: 2. Furthermore, the modified S-protein may comprise one or more than one amino acid substitution corresponding to amino acids at positions 667, 668, 670, or a combination thereof, when compared to reference amino acid sequence of SEQ ID NO: 2. Accordingly, the modified S-protein may comprise substitutions that correspond to amino acids at positions 667, 668 and 670, when compared to reference amino acid sequence of SEQ ID NO: 2.

[0043] In one example, the one or more than one substitution may correspond to amino acids at positions 667, 668, 670, 971 and 972, when compared to reference amino acid sequence of SEQ ID NO: 2. The substitution of the amino acid corresponding to the amino acid at position 667 of SEQ ID NO: 2 may be to glycine or a conserved

substitution of glycine, the substitution of the amino acid corresponding to position 668 of SEQ ID NO: 2 may be to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to position 670 of SEQ ID NO: 2 may be to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to the amino acid at position 971 of SEQ ID NO: 2 may be to a proline or a conserved substitution of proline and the substitution of the amino acid corresponding to the amino acid at position 972 of SEQ ID NO: 2 may be to a proline or a conserved substitution of proline. The modified S-protein as described above may further comprises an amino acid substitution corresponding to amino acid at position 923, when compared to reference amino acid sequence of SEQ ID NO: 2.

[0044] In another example the one or more than one amino acid substitution may correspond to amino acids at positions 667, 668, 670, 802, 927, 971 and 972, when compared to reference amino acid sequence of SEQ ID NO: 2. The substitution of the amino acid corresponding to the amino acid at position 667 of SEQ ID NO: 2 may be to glycine or a conserved substitution of glycine, the substitution of the amino acid corresponding to position 668 of SEQ ID NO: 2 may be to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to position 670 of SEQ ID NO: 2 may be to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to the amino acid at positions 802 of SEQ ID NO: 2 may be to a proline or a conserved substitution of proline, the substitution of the amino acid corresponding to the amino acid at positions 927 of SEQ ID NO: 2 may be to a proline or a conserved substitution of proline, the substitution of the amino acid corresponding to the amino acid at positions 971 of SEQ ID NO: 2 may be to a proline or a conserved substitution of proline and the substitution of the amino acid corresponding to the amino acid at positions 972 of SEQ ID NO: 2 may be to a proline or a conserved substitution of proline.

[0045] In another example the modified S-protein as described above may further comprises an amino acid substitution corresponding to amino acid at position 923, when compared to reference amino acid sequence of SEQ ID NO: 2. The substitution in the modified S-protein of the amino acid corresponding to the amino acid at position 667 of

SEQ ID NO: 2 may be to glycine or a conserved substitution of glycine, the substitution of the amino acid corresponding to position 668 of SEQ ID NO: 2 may be to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to position 670 of SEQ ID NO: 2 may be to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to the amino acid at positions 802, 927, 971 and 972 of SEQ ID NO: 2 may be to a proline or a conserved substitution of proline and the substitution of the amino acid corresponding to position 923 of SEQ ID NO: 2 may be to phenylalanine or a conserved substitution of phenylalanine.

[0046] The modified the S-protein may comprise from 80% to 100% identity with amino acids of SEQ ID NO: 5, 21, 30, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 95, 96, 97, 108, 109, 110, 144, 145, 146, 155, 156 or 157, or with amino acids 24-1259 of SEQ ID NO: 47 amino acids 25-1259 of SEQ ID NO: 48, amino acids 25-1259 of SEQ ID NO: 49, amino acids 25-1259 of SEQ ID NO: 50, amino acids 25-1259 of SEQ ID NO: 51, amino acids 25-1259 of SEQ ID NO: 52, amino acids 25-1259 of SEQ ID NO: 53, amino acids 25-1259 of SEQ ID NO: 54, amino acids 25-1259 of SEQ ID NO: 55, amino acids 25-1259 of SEQ ID NO: 56, amino acids 25-1259 of SEQ ID NO: 57, amino acids 25-1259 of SEQ ID NO: 58, amino acids 25-1262 of SEQ ID NO: 59, amino acids 25-1261 of SEQ ID NO: 60, amino acids 25-1258 of SEQ ID NO: 61, or amino acids 25-1256 of SEQ ID NO: 62, amino acids 25-1243 of SEQ ID NO: 95, amino acids 25-1240 of SEQ ID NO: 96, amino acids 25-1243 of SEQ ID NO: 97, amino acids 25-1341 of SEQ ID NO: 108, amino acids 25-1338 of SEQ ID NO: 109, amino acids 25-1341 of SEQ ID NO: 110, amino acids 25-1351 of SEQ ID NO: 144, amino acids 25-1348 of SEQ ID NO: 145, amino acids 25-1351 of SEQ ID NO: 146, amino acids 25-1159 of SEQ ID NO: 155, amino acids 25-1156 of SEQ ID NO: 156, or amino acids 25-1159 of SEQ ID NO: 157.

[0047] In another example, it is provided a nucleic acid that comprises a nucleotide sequence that encodes the modified S-protein as described above.

[0047a] In another aspect, the present disclosure refers to a nucleic acid comprising a nucleotide sequence encoding the modified S protein as disclosed herein.

[0048] In a further example a composition comprising an effective dose of the modified S-protein, the trimer comprising the modified S-protein or VLP comprising the modified S-protein as described above and a pharmaceutically acceptable carrier, adjuvant, vehicle or

excipient is provided. In yet another example, it is provided a vaccine for inducing an immune response. The vaccine comprises an effective dose of the modified S protein, the trimer comprising the modified S-protein or VLP comprising the modified S-protein as described above as described above.

[0048a] In another aspect, the present disclosure refers to a composition comprising an effective dose of the modified S-protein as disclosed herein, the trimer as disclosed herein or the VLP as disclosed herein and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

[0048b] In another aspect, the present disclosure refers to a vaccine for inducing an immune response, the vaccine comprising an effective dose of the modified S-protein as disclosed herein, the trimer as disclosed herein, the VLP as disclosed herein, or the composition as disclosed herein.

[0049] The composition further comprises a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient. In a further example, it is provided a vaccine for inducing an immune response. The vaccine comprises an effective dose of the VLP comprising a modified coronavirus as described above. The vaccine may be a multivalent vaccine, comprising a mixture of VLP.

[0050] In yet another example a (non-human) host or host cell comprising the modified S-protein, trimer or VLP as described above is provided. In yet another example a host or host cell comprising the VLP as described above is provided. In another example it is provided a composition comprising an effective dose of the VLP comprising the modified S-protein as described above is provided.

[0051] In yet another example, a S-protein trimer is provided. The trimer comprises modified coronavirus S-protein, the modified S-protein comprising

- an ectodomain derived from a coronavirus S-protein,

- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:

- a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein, and

- a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein. The modified S-protein in the trimer may comprise one or more than one amino acid substitution when compared to a wild-type coronavirus amino acid sequence, as described above. In a further example a composition comprising an effective dose of the trimer as described above and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient is provided. In another example, virus like particle (VLP) comprising the trimer as described above are also provided. The VLP may further comprise plant lipids. In another example it is provided a composition comprising

an effective dose of the VLP comprising the trimer as described above and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient is provided. In a further example, it is provided a vaccine for inducing an immune response. The vaccine comprises an effective dose of the trimer as described above. In a further example, it is provided a vaccine for inducing an immune response. The vaccine comprises an effective dose of the VLP comprising the trimer as described above. The vaccine may be a multivalent vaccine, comprising a mixture of VLP. In yet another example a non-human host or host cell comprising the trimer or the VLP comprising the trimer as described above is provided.

[0051a] In another aspect, the present disclosure refers to a non-human host or host cell comprising the modified S-protein as disclosed herein, the trimer as disclosed herein, the nucleic acid as disclosed herein or the VLP as disclosed herein.

[0052] In another example, it is provided a method for inducing immunity to a Coronavirus infection in a subject, the method comprising administering the composition or vaccines as described above. The composition or vaccine may be administered once to the subject or the composition or vaccine may be administered multiple times to the subject. The composition or vaccine may be administered as an initial dose and one or more than one subsequent doses may be administered between 1 day and 6 month from the administration of the initial dose. The subsequent dose may be administered after 21 days from the administration of the initial dose.

[0052a] In another aspect, the present disclosure refers to use of the composition as disclosed herein or the vaccine as disclosed herein in the manufacture of a medicament for inducing immunity to a Coronavirus infection in a subject.

[0053] In another aspect an antibody or antibody fragment prepared using the composition or vaccine as described above are provided.

[0053a] In another aspect, the present disclosure refers to a method of producing a modified S-protein in a non-human host or host cell comprising: a) introducing the nucleic acid as disclosed herein into the non-human host or host cell, or providing the non-human host or host cell comprising the nucleic acid as disclosed herein, and b) incubating the non-human host or host cell under conditions that permit the expression of the nucleic acid, thereby producing the modified S-protein.

[0054] In yet another example, it is provided A) a method of producing a virus like particle (VLP) in a (non-human) host or host cell comprising:

a) introducing into a non-human host or host cell the nucleic acid comprising a nucleotide sequence that encodes the modified S-protein as described above; or providing the non-human host or host cell comprising the nucleic acid comprising a nucleotide sequence that encodes the modified S-protein as described above, and

b) incubating the non-human host or host cell under conditions that permit the expression of the nucleic acid, thereby producing the VLP.

[0054a] In another aspect, the present disclosure refers to a method of producing a virus like particle (VLP) in a non-human host or host cell comprising: a) introducing the nucleic acid as disclosed herein into the non-human host or host cell, or providing the non-human host or host cell comprising the nucleic acid as disclosed herein, and b) incubating the non-human host or host cell under conditions that permit the expression of the nucleic acid, thereby producing the VLP.

[0055] In a further step c) the non-human host or host cell may be harvested.

[0056] In a further example, it is provided B) a method of increasing yield of production of a Coronavirus S-protein in a (non-human) host or host cell comprising:

a) introducing the nucleic acid comprising a nucleotide sequence that encodes the modified S-protein as described above into the non-human host or host cell; or providing a non-human host or host cell comprising the nucleic acid comprising a nucleotide sequence that encodes the modified S-protein as described above; and

b) incubating the non-human host or host cell under conditions that permit expression of the modified S-protein encoded by the nucleic acid, thereby producing modified S-protein at a higher yield compared to a host or host cell expressing the unmodified S-protein under similar or identical conditions.

[0056a] In another aspect, the present disclosure refers to a method of increasing yield of production of a modified Coronavirus S-protein in a non-human host or host cell comprising: a) introducing the nucleic acid as disclosed herein into the host or host cell; or providing a non-human host or host cell comprising the nucleic acid as disclosed herein; and b) incubating the non-human host or host cell under conditions that permit expression of the modified S-protein encoded by the nucleic acid, thereby producing modified S-protein at a higher yield compared to a host or host cell expressing the unmodified S-protein.

[0057] In a further step c) the non-human host or host cell may be harvested.

[0058] In yet another example, it is provided C) a method of increasing yield of production of virus like particle (VLP) in a (non-human) host or host cell comprising:

a) introducing into the non-human host or host cell, a nucleic acid encoding a modified coronavirus S-protein comprising

- an ectodomain derived from a coronavirus S-protein,

- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:

    - a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and

    - a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein;

providing a non-human host or host cell comprising the nucleic acid encoding the modified S-protein comprising

- an ectodomain derived from a coronavirus S-protein,

- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:

- a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and

- a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; and

b) incubating the non-human host or host cell under conditions that permit expression of the modified S-protein encoded by the nucleic acid, thereby producing VLP comprising modified S-protein at a higher yield compared to the yield of VLP in a host of host cell that expresses unmodified S protein under similar or identical conditions.

[0058a] In another aspect, the present disclosure refers to a method of increasing yield of production of virus like particle (VLP) in a non-human host or host cell comprising: a) introducing into a non-human host or host cell a nucleic acid encoding a modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein, or providing a non-human host or host cell comprising the nucleic acid encoding the modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; and b) incubating the non-human host or host cell under conditions that permit expression of the modified S-protein encoded by the nucleic acid, thereby producing VLP comprising modified S-protein at a higher yield compared to the yield of VLP in a host of host cell that expresses unmodified S protein.

[0059] In a further example, it is provided D) a method of producing a virus like particle (VLP) in a (non-human) host or host cell comprising:

a) introducing into a non-human host or host cell, a nucleic acid encoding a modified coronavirus S-protein comprising

- an ectodomain derived from a coronavirus S-protein,

- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:

- a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and

- a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; or

providing a non-human host or host cell comprising the nucleic acid encoding the modified S-protein comprising

- an ectodomain derived from a coronavirus S-protein,
- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:
    - a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and
    - a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; and

b) incubating the non-human host or host cell under conditions that permit the expression of the nucleic acid, thereby producing the VLP.

[0059a] In another aspect, the present disclosure refers to a method of producing a virus like particle (VLP) in a non-human host or host cell comprising: a) introducing into the a non-human host or host cell a nucleic acid encoding a modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; or providing a non-human host or host cell comprising the nucleic acid encoding the modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; and

b) incubating the non-human host or host cell under conditions that permit the expression of the nucleic acid, thereby producing the VLP.

[0060] In a further example the VLP of method A), B), C) or D) may further be extracted and purified from the host or host cell. The host or host cell may comprise a plant, a plant cell, a fungi, a fungi cell, an insect, an insect cell, an animal or an animal cell. The host or host cell of method A), B), C) or D) may be a plant, portion of a plant or plant cell.

[0061] In another aspect it is provided a VLP produced by the method of A), B), C) or D). In another aspect, the present disclosure refers to a modified S-protein produced by the method as disclosed herein.

[0062] Furthermore, in yet another example it is provided a composition comprising an adjuvant and virus-like particles (VLP), the VLP comprising modified coronavirus S-protein, the modified S-protein comprising

- an ectodomain derived from a coronavirus S-protein,
- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:
    - a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and
    - a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein and wherein the modified S-

protein further comprises substitutions at positions 667, 668, 670, 971 and 972 when compared to reference amino acid sequence of SEQ ID NO: 2.

[0062a] In another aspect, the present disclosure refers to a composition comprising a virus-like particles (VLP), the VLP comprising a modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein and wherein the S-protein comprises substitutions at positions 667, 668, 670, 971 and 972 when compared to reference amino acid sequence of SEQ ID NO: 2.

[0063] In yet another example it is provided a composition comprising virus-like particle (VLP), the VLP comprising modified coronavirus S-protein, the modified S protein comprising

- an ectodomain derived from a coronavirus S-protein,

- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:

  - a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and

  - a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; and wherein the modified S-protein comprises a glycine substitution at position 667, a serine substitution at position 668, a serine substitution at position 670, a proline substitution at position 971 and a proline substitution at position 972, the position corresponding to reference amino acid sequence of SEQ ID NO: 2. The influenza hemagglutinin (HA) protein may be derived from influenza type B or influenza subtype H1, H3, H5, H6, H7 or H9. The composition may further comprise an adjuvant.

[0063a] In another aspect, the present disclosure refers to a composition comprising a virus-like particle (VLP), the VLP comprising a modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein and wherein the S-protein comprises a glycine substitution at position 667, a serine substitution at position 668, a serine substitution at position 670, a proline substitution at position 971 and a proline substitution at position 972, the position corresponding to reference amino acid sequence of SEQ ID NO: 2.

[0064] In a further example it is provided a composition comprising virus-like particle (VLP), the VLP comprising modified coronavirus S-protein, the modified S protein comprising the sequence of SEQ ID NO: 21. The composition may further comprise an adjuvant.

[0064a] In another aspect, the present disclosure refers to a composition comprising a virus-like particle (VLP), the VLP comprising a modified coronavirus S-protein, the modified S-protein comprising the sequence of SEQ ID NO: 21 or amino acids 25-1259 of SEQ ID NO: 51.

[0064b] In another aspect, the present disclosure refers to a composition comprising a virus-like particle (VLP), the VLP comprising modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein and wherein the S-protein comprises substitutions at positions 667, 668, 670, 802, 923, 927, 971 and 972 when compared to reference amino acid sequence of SEQ ID NO: 2.

[0065] This summary of the disclosure does not necessarily describe all features of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0066] These and other features of the invention will become more apparent from the following description in which reference is made to the appended drawings wherein:

WHAT IS CLAIMED IS:

1. A modified coronavirus S-protein comprising, in series,

- an ectodomain derived from a coronavirus S-protein,

- a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising:

    - a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and

    - a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein.

2. The modified coronavirus S-protein of claim 1, wherein the TM is directly fused to the CT.

3. The modified coronavirus S-protein of claim 1, wherein the TM is a chimeric TM comprising a N-terminal sequence derived from the coronavirus S-protein TM and a C-terminal sequence derived from the influenza HA protein TM.

4. The modified coronavirus S-protein of claim 3, wherein the chimeric TM comprises a N-terminal sequence derived from the coronavirus S-protein TM comprising at least 20 amino acids corresponding to amino acids 1-20 of SEQ ID NO: 18 or SEQ ID NO: 169, or at least 21 amino acids corresponding to amino acids 1-21 of SEQ ID NO: 118 or 164, or at least 22 amino acids corresponding to amino acids 1-22 of SEQ ID NO: 123 and one or more than one amino acid from the C-terminal end of the influenza HA protein TM.

5. The modified coronavirus S-protein of claim 4, wherein the one or more than one amino acid from the C-terminal end of the influenza HA protein TM are selected from AGL or conserved substitution of AGL, MAGL or conserved substitution of MAGL.

6. The modified coronavirus S-protein of claim 1, wherein the CT is a chimeric CT comprising a N-terminal sequence derived from the coronavirus S-protein CT and a C-terminal sequence derived from the influenza HA protein CT.

7. The modified coronavirus S-protein of claim 6, wherein the chimeric CT comprises a C-terminal sequence derived from the influenza HA protein CT comprising amino acids corresponding to amino acids 27-37 of SEQ ID NO: 18, 126, 128, 129, 130 or 131; or amino acids corresponding to amino acids 27-36 of SEQ ID NO: 127 and one or more than one amino acid from the N-terminal end of the coronavirus S-protein CT.

8. The modified coronavirus S-protein of claim 7, wherein the one or more than one amino acid from the N-terminal end of the coronavirus S-protein CT are selected from C or a conserved substitution of C, CC or a conserved substitution of CC, or CCM or a or a conserved substitution of CCM.

9. The modified coronavirus S-protein of claim 3, wherein the chimeric TM comprises amino acids corresponding to amino acids 1-20 of SEQ ID NO: 18 or SEQ ID NO: 169, or to amino acids 1-21 of SEQ ID NO: 118 or SEQ ID NO: 164, or amino acids 1-22 of SEQ ID NO: 123.

10. The modified coronavirus S-protein of claim 4, wherein the chimeric CT comprises amino acids corresponding to amino acids of 27-37 of SEQ ID NO: 18, 126, 128, 129, 130 or 131; or amino acids 27-36 of SEQ ID NO: 127.

11. The modified coronavirus S-protein of claim 1, wherein the chimeric TMCT comprises a chimeric TM comprising amino acids corresponding to amino acids 1-20 of SEQ ID NO: 18 or SEQ ID NO: 169, or to amino acids 1-21 of SEQ ID NO: 118 or SEQ ID NO: 164, or amino acids 1-22 of SEQ ID NO: 123 and, a chimeric CT comprising amino acids corresponding to amino acids 27-37 of SEQ ID NO: 18, 126, 128, 129, 130 or 131; amino acids 27-36 of SEQ ID NO: 127 or a combination thereof.

12. The modified S-protein of any one of claims 1-11, wherein the S-protein comprises one or more than one amino acid substitution when compared to a wild-type coronavirus S-protein amino acid sequence.

13. The modified S-protein of claim 12, wherein the one or more than one amino acid substitution comprises:
i) substitutions that restricts the processing at a cleavage site between S1 and S2 subunit,
ii) substitution of one or more than one amino acid to one or more than one proline, or
iii) substitutions that restrict the processing at the cleavage site between the S1 and the S2 subunit and substitution of one or more than one amino acid to one or more than one proline.

14. The modified S-protein of claim 12 or 13, wherein the one or more than one amino acid substitution correspond to amino acids at positions 971 and 972, when compared to reference amino acid sequence of SEQ ID NO: 2.

15. The modified S-protein of claim 12 or 13, wherein the one or more than one amino acid substitution correspond to amino acids at positions 802, 927, 971 and 972, when compared to reference amino acid sequence of SEQ ID NO: 2.

16. The modified S-protein of claim 14 or 15, wherein the modified S-protein further comprises one or more than one amino acid substitution corresponding to amino acids at positions 667, 668, 670, or a combination thereof, when compared to reference amino acid sequence of SEQ ID NO: 2.

17. The modified S-protein of claim 16, wherein the substitution of the amino acid corresponding to the amino acid at position 667 of SEQ ID NO: 2 is to glycine or a conserved substitution of glycine, the substitution of the amino acid corresponding to position 668 of SEQ ID NO: 2 is to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to position 670 of SEQ ID NO: 2 is to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to the amino acid at positions 971 and 972 of SEQ ID NO: 2 is to a proline or a conserved substitution of proline.

18. The modified S-protein of claim 16, wherein the substitution of the amino acid corresponding to the amino acid at position 667 of SEQ ID NO: 2 is to glycine or a conserved substitution of glycine, the substitution of the amino acid corresponding to

position 668 of SEQ ID NO: 2 is to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to position 670 of SEQ ID NO: 2 is to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to the amino acid at positions 802, 927, 971 and 972 of SEQ ID NO: 2 is to a proline or a conserved substitution of proline.

19. The modified S-protein of any one of claims 14-18, wherein the modified S-protein further comprises an amino acid substitution corresponding to amino acid at position 923, when compared to reference amino acid sequence of SEQ ID NO: 2.

20. The modified S-protein of claim 19, wherein the substitution of the amino acid corresponding to the amino acid at position 667 of SEQ ID NO: 2 is to glycine or a conserved substitution of glycine, the substitution of the amino acid corresponding to position 668 of SEQ ID NO: 2 is to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to position 670 of SEQ ID NO: 2 is to a serine or a conserved substitution of serine, the substitution of the amino acid corresponding to the amino acid at positions 802, 927, 971 and 972 of SEQ ID NO: 2 is to a proline or a conserved substitution of proline and the substitution of the amino acid corresponding to position 923 of SEQ ID NO: 2 is to phenylalanine or a conserved substitution of phenylalanine.

21. The modified S-protein of any one of claims 12-20, wherein the one or more than one substitution maintains the S-protein in a pre-fusion state or produces are higher yield of the modified S-protein when expressed in a host or host cell, when compared to the yield of a corresponding S-protein without the one or more than one substitutions expressed in the host or host cell.

22. The modified S-protein of any one of claims 1-21, wherein the S-protein comprises an S1 subunit and an S2 subunit, wherein the S2 subunit comprises the chimeric TMCT.

23. The modified S-protein of any one of claims 1-22, wherein the influenza HA protein is derived from influenza type B or influenza subtype H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15, or H16.

24. The modified S-protein of any one of claims 1-23, wherein the coronavirus S-protein is derived from SARS-CoV-2, SARS-CoV-1, MERS-CoV, OC43-CoV or 229E-CoV.

25. The modified S-protein of any one of claims 1-23, wherein the ectodomain is derived from SARS-CoV-2, SARS-CoV-1, MERS-CoV, OC43-CoV or 229E-CoV, the TM or the portion of the TM is derived from SARS-CoV-2, SARS-CoV-1, MERS-CoV, OC43-CoV or 229E-CoV or wherein the ectodomain and the TM or the portion of the TM are derived from SARS-CoV-2, SARS-CoV-1, MERS-CoV, OC43-CoV or 229E-CoV.

26. The modified S-protein of any one of claims 1-25, wherein the TMCT comprises a sequence having about 80% to about 100% identity with the sequence of SEQ ID NO: 18, 19, 37, 38, 39, 64, 126, 127, 128, 129, 130, 131, 118, 119, 120, 123, 124, 125, 134, 135, 164, 165, 166, 169, 170, 171, 172 or 173.

27. The modified S-protein of any one of claims 1-26, wherein the modified S-protein is produced as a precursor protein, the precursor protein comprising the modified S-protein and a signal peptide.

28. The modified S-protein of claim 27, wherein the signal peptide is native or non-native to the modified S-protein.

29. The modified S-protein of claim 27 or 28, wherein the signal peptide is derived from the signal peptide of protein disulfide-isomerase (PDI).

30. The modified S-protein of any one of claims 1-29, wherein the modified S-protein comprises plant specific N-glycans.

31. The modified S-protein of claim 1, wherein the amino acid sequence of the S-protein comprises from 80% to 100% identity with amino acids of SEQ ID NO: 5, 21, 30, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 95, 96, 97, 108, 109, 110, 144, 145, 146, 155, 156 or 157, or with amino acids 24-1259 of SEQ ID NO: 47 amino acids 25-1259 of SEQ ID NO: 48, amino acids 25-1259 of SEQ ID NO: 49, amino acids 25-1259 of SEQ ID NO: 50, amino acids 25-1259 of SEQ ID NO: 51, amino acids 25-1259 of SEQ ID NO: 52, amino acids 25-1259 of SEQ ID NO: 53, amino acids 25-1259 of SEQ

ID NO: 54, amino acids 25-1259 of SEQ ID NO: 55, amino acids 25-1259 of SEQ ID NO: 56, amino acids 25-1259 of SEQ ID NO: 57, amino acids 25-1259 of SEQ ID NO: 58, amino acids 25-1262 of SEQ ID NO: 59, amino acids 25-1261 of SEQ ID NO: 60, amino acids 25-1258 of SEQ ID NO: 61, or amino acids 25-1256 of SEQ ID NO: 62, amino acids 25-1243 of SEQ ID NO: 95, amino acids 25-1240 of SEQ ID NO: 96, amino acids 25-1243 of SEQ ID NO: 97, amino acids 25-1341 of SEQ ID NO: 108, amino acids 25-1338 of SEQ ID NO: 109, amino acids 25-1341 of SEQ ID NO: 110, or amino acids 25-1351 of SEQ ID NO: 144, amino acids 25- 1348 of SEQ ID NO: 145, amino acids 25-1351 of SEQ ID NO: 146, amino acids 25- 1159 of SEQ ID NO: 155, amino acids 25-1156 of SEQ ID NO: 156, or amino acids 25- 1159 of SEQ ID NO: 157.

32. The modified S-protein of any one of claims 1-30, wherein the CT or portion of the CT comprises from 80% to 100% identity with the sequence of SEQ ID NO: 15, or with amino acids 35-50 of SEQ ID NO 6, 8, 7, 9, 10, 12, 13 or 14, , or with amino acids 34-49 of SEQ ID NO 11, or with amino acids 553-568 of SEQ ID NO:3 or with amino acids 22-37 of SEQ ID NO:18, or with amino acids 21-40 of SEQ ID NO: 19, or with amino acids 21-39 of SEQ ID NO: 37, or with amino acids 25-36 of SEQ ID NO: 38 or with amino acids 24-34 of SEQ ID NO: 39, or amino acids 22-37 of SEQ ID NO: 126, 128, 129, 130 or 131 or amino acids 22-36 of SEQ ID NO: 127

33. The modified S-protein of claim 27, wherein the precursor protein comprises from 80% to 100% identity with amino acids 1-1234 of SEQ ID 1, or with amino acids 1-1234 of SEQ ID NO: 5, with amino acids 1-1243 of SEQ ID NO: 30, with amino acids 1-1227 of SEQ ID NO: 95 , with amino acids 1-1325 of SEQ ID NO: 108, with amino acids 1-1216 of SEQ ID NO: 112, with amino acids 1-1318 of SEQ ID NO: 113, with amino acids 1-1335 of SEQ ID NO: 144, with amino acids 1-1143 of SEQ ID NO: 155, with amino acids 1-1325 of SEQ ID NO: 158 , with amino acids 1-1135 of SEQ ID NO: 159, and wherein the amino acid sequence of the CT comprises from 80% to 100% identity with the sequence of SEQ ID NO: 15, or with amino acids 35-50 of SEQ ID NO 6, 8, 7, 9, 10, 12, 13 or 14, or with amino acids 34-49 of SEQ ID NO 11, or with amino acids 553-568 of SEQ ID NO:3.

34. A nucleic acid comprising a nucleotide sequence encoding the modified S protein of any one of claims 1-33.

35. A trimer comprising the modified S-protein of any one of claims 1-33.

36. A virus like particle (VLP) comprising the modified S-protein of any one of claims 1-33 or the trimer of claim 35.

37. The VLP of claim 36, wherein the VLP further comprises plant lipids.

38. A composition comprising an effective dose of the modified S-protein of any one of claims 1-33, the trimer of claim 35 or the VLP of any one of claims 36-37 and a pharmaceutically acceptable carrier, adjuvant, vehicle or excipient.

39. A vaccine for inducing an immune response, the vaccine comprising an effective dose of the modified S-protein of any one of claims 1-33, the trimer of claim 35, the VLP of any one of claims 36-37, or the composition of claim 38.

40. The vaccine of claim 39, wherein the vaccine is a multivalent vaccine, comprising a mixture of VLP.

41. Use of the composition of claim 38 or the vaccine of claim 39 or 40 in the manufacture of a medicament for inducing immunity to a Coronavirus infection in a subject.

42. The use of claim 41, wherein the composition or vaccine is to be administered once to the subject.

43. The use of claim 41, wherein the composition or vaccine is to be administered multiple times to the subject.

44. The use of claim 41, wherein the composition or vaccine is to be administered as an initial dose and one or more than one subsequent doses are to be administered between 1 day and 6 months from the administration of the initial dose.

45. An antibody or antibody fragment prepared using the composition of claim 38 or the vaccine of claim 39 or 40.

46. A non-human host or host cell comprising the modified S-protein of any one of claims 1-33, the trimer of claim 35, the nucleic acid of claims 34 or the VLP of claims 36 or 37.

47. A method of producing a modified S-protein in a non-human host or host cell comprising:

a) introducing the nucleic acid of claim 34 into the non-human host or host cell, or providing the non-human host or host cell comprising the nucleic acid of claim 34, and

b) incubating the non-human host or host cell under conditions that permit the expression of the nucleic acid, thereby producing the modified S-protein.

48. A method of producing a virus like particle (VLP) in a non-human host or host cell comprising:

a) introducing the nucleic acid of claim 34 into the non-human host or host cell, or providing the non-human host or host cell comprising the nucleic acid of claim 34, and

b) incubating the non-human host or host cell under conditions that permit the expression of the nucleic acid, thereby producing the VLP.

49. The method of claim 47 or 48, the method further comprising step c) of harvesting the non-human host or host cell.

50. A method of increasing yield of production of a modified Coronavirus S-protein in a non-human host or host cell comprising:

a) introducing the nucleic acid of claims 34 into the host or host cell; or providing a non-human host or host cell comprising the nucleic acid of claim 34; and

b) incubating the non-human host or host cell under conditions that permit expression of the modified S-protein encoded by the nucleic acid, thereby producing modified S-protein at a higher yield compared to a host or host cell expressing the unmodified S-protein.

51. A method of increasing yield of production of virus like particle (VLP) in a non-human host or host cell comprising:

a) introducing into a non-human host or host cell a nucleic acid encoding a modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein, or

providing a non-human host or host cell comprising the nucleic acid encoding the modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; and

b) incubating the non-human host or host cell under conditions that permit expression of the modified S-protein encoded by the nucleic acid, thereby producing VLP comprising modified S-protein at a higher yield compared to the yield of VLP in a host of host cell that expresses unmodified S protein.

52. A method of producing a virus like particle (VLP) in a non-human host or host cell comprising:

a) introducing into the a non-human host or host cell a nucleic acid encoding a modified coronavirus S-protein comprising, in series, an ectodomain derived

from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; or

providing a non-human host or host cell comprising the nucleic acid encoding the modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein; and

b) incubating the non-human host or host cell under conditions that permit the expression of the nucleic acid, thereby producing the VLP.

53. The method of any one of claims 48, 51 or 52, wherein the VLP is further extracted and purified from the non-human host or host cell.

54. A VLP produced by the method of claim 48, 51 or 52.

55. A modified S-protein produced by the method of claim 47 or 50.

56. The method of any one of claims 47-55, or the non-human host or host cell of claim 46, wherein the non-human host or host cell comprises a plant, portion of a plant, a plant cell, a fungi, a fungi cell, an insect, an insect cell, an animal or an animal cell.

57. A composition comprising a virus-like particles (VLP), the VLP comprising a modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin

(HA) protein and wherein the S-protein comprises substitutions at positions 667, 668, 670, 971 and 972 when compared to reference amino acid sequence of SEQ ID NO: 2.

58. A composition comprising a virus-like particle (VLP), the VLP comprising a modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein and wherein the S-protein comprises a glycine substitution at position 667, a serine substitution at position 668, a serine substitution at position 670, a proline substitution at position 971 and a proline substitution at position 972, the position corresponding to reference amino acid sequence of SEQ ID NO: 2.

59. A composition comprising a virus-like particle (VLP), the VLP comprising a modified coronavirus S-protein, the modified S-protein comprising the sequence of SEQ ID NO: 21 or amino acids 25-1259 of SEQ ID NO: 51.

60. A composition comprising a virus-like particle (VLP), the VLP comprising modified coronavirus S-protein comprising, in series, an ectodomain derived from a coronavirus S-protein, a transmembrane and cytosolic tail domain (TMCT), wherein the TMCT is a chimeric TMCT, comprising: a transmembrane domain (TM), wherein the TM or a portion of the TM is derived from a coronavirus S-protein and a cytosolic tail (CT), wherein the CT or a portion of the CT is derived from an influenza hemagglutinin (HA) protein and wherein the S-protein comprises substitutions at positions 667, 668, 670, 802, 923, 927, 971 and 972 when compared to reference amino acid sequence of SEQ ID NO: 2.