

RECOMBINANT VIRUS-LIKE PARTICLES

RELATED APPLICATION DATA

The present application claims priority from United States Patent Application No. 63/510,970 filed 29 June 2023 entitled “Recombinant virus-like particles”, the entire contents of which is hereby incorporated by reference.

SEQUENCE LISTING

The present application is filed together with a Sequence Listing in electronic form. The entire contents of the Sequence Listing are hereby incorporated by reference.

FIELD

The present disclosure relates to a recombinant virus-like particle (VLP) comprising an antigen for use as a vaccine. In an aspect, the present disclosure relates to a recombinant VLP comprising a capsid fusion protein for use as a vaccine.

BACKGROUND

Respiratory viral infections are a significant threat to human health. Infections, such as those caused by the influenza virus and severe acute respiratory syndrome coronavirus (SARS-CoV) have been known to cause global pandemics, killing millions of people worldwide. Recently, SARS-CoV-2 has been responsible for causing the on-going worldwide pandemic of the severely infectious coronavirus disease 2019 (COVID-19). Moreover, respiratory syncytial virus (RSV) is the single most common cause of respiratory hospitalization in infants, and reinfection remains common in later life. Whilst some vaccines are available for viral infections such as influenza, SARS-CoV-2 and RSV, further improvements can be made to increase their efficacy and/or improve treatment strategies.

Currently, egg-based manufacturing processes are the most common way that vaccines are produced. This process requires a significant amount of time to optimize virus growth in the eggs, as well as resources (i.e., eggs) to produce sufficient amounts of vaccine, particularly during a pandemic. Furthermore, given the long development time required, vaccine strain selection is conducted before the vaccine is made available, making it difficult to respond to changes in the virus. Vaccines have also been produced using cell-based manufacturing processes involving cultured mammalian cells (e.g. Madin-Darby Canine Kidney, or MDCK cells) in place of eggs, and viral-based platforms involving recombinant virus (e.g. baculovirus encoding an antigen of influenza) have also been utilised.

There remains a need for the development of specific and efficient viral vaccines that can be produced more rapidly and with broader utility than current egg-based techniques, for the treatment or prevention of respiratory viral infections, such as influenza, RSV and SARS-CoV-

2. Nucleic acid-based vaccines offer distinct advantages over the current egg-based manufacturing platform, although some challenges remain. For example, the inherently labile nature of mRNA results in most RNA-based vaccines having limited ability to provide antigen at a dose and duration required to produce a strong, durable immune response.

Therefore, it will be apparent to the skilled person that there is a need in the art for compositions with broader utility and/or improved efficacy that are suitable for use as vaccines.

SUMMARY

The present disclosure is based on the inventors' identification of recombinant virus-like particles (VLPs) comprising a capsid fusion protein and a lipid bilayer that are suitable for the treatment of a disease, condition or infection, such as a SARS-CoV-2 infection, influenza or coronavirus disease 2019 (COVID-19). The findings by the inventors therefore provide basis for methods of treating or preventing or delaying progression of a disease, condition or infection, such as a SARS-CoV-2 infection or COVID-19, as well as complications thereof including pneumonia and acute respiratory distress syndrome (ARDS)) in a subject. The lipid bilayer present in the VLPs described in the present disclosure provides for a technical advantage by preventing an immune response in the subject to the capsid protein that forms part of the capsid fusion protein. Further, the VLP constructs provided by the present disclosure are capable of assembling into VLPs and providing immunogenicity in the form of a vaccine.

Accordingly, the present disclosure provides a recombinant virus-like particle (VLP) comprising a capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:

- (a) a capsid protein from a non-enveloped virus;
- (b) a transmembrane (TM) protein domain; and
- (c) an antigen protein,

wherein the capsid protein is encapsulated within the lipid bilayer.

The present disclosure also provides a virus-like particle (VLP) comprising a capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:

- (a) a capsid protein from a non-enveloped virus; and
- (b) an antigen protein,

wherein the capsid protein is encapsulated within the lipid bilayer.

In one example, the capsid protein from a non-enveloped virus is from an alfalfa mosaic virus (AMV), bacteriophage MS2 or bacteriophage AP205.

In one example, the capsid protein is from AMV. In some examples, the capsid protein comprises the amino acid sequence of SEQ ID NO: 32, or an amino acid sequence at least 90% identical to SEQ ID NO: 32.

In one example, the capsid protein is from bacteriophage MS2. In some examples, the capsid protein comprises the amino acid sequence of SEQ ID NO: 35, or an amino acid sequence at least 90% identical to SEQ ID NO: 35.

In some examples, the capsid protein is a dimer capsid protein from bacteriophage MS2. In some examples, the capsid protein comprises the amino acid sequence of SEQ ID NO: 36, or an amino acid sequence at least 90% identical to SEQ ID NO: 36. In some examples, the capsid protein comprises the amino acid sequence of SEQ ID NO: 37, or an amino acid sequence at least 90% identical to SEQ ID NO: 37.

In one example, the capsid protein is from bacteriophage AP205. In some examples, the capsid protein comprises the amino acid sequence of SEQ ID NO: 33, or an amino acid sequence at least 90% identical to SEQ ID NO: 33.

In some examples, the capsid protein is a dimer capsid protein from bacteriophage AP205. In some examples, the capsid protein comprises the amino acid sequence of SEQ ID NO: 34, or an amino acid sequence at least 90% identical to SEQ ID NO: 34.

In one example, the capsid protein of the capsid fusion protein is a trimer or a dimer.

In one example, the capsid fusion protein further comprises a signal sequence. In some examples, the signal sequence comprises the amino acid sequence of SEQ ID NO: 21, or an amino acid sequence at least 90% identical to SEQ ID NO: 21.

In one example, the capsid fusion protein further comprises a peptide tag such as a polyhistidine tag. In one embodiment the peptide tag is His6. In some examples, the polyhistidine tag comprises the amino acid sequence of SEQ ID NO: 28, or an amino acid sequence at least 90% identical to SEQ ID NO: 28.

In one example, the capsid protein is fused to the TM protein domain by a peptide linker. In this example, the linker is a short protein of up to about 30 amino acids, such as about 5-30 amino acids, about 5-25 amino acids, about 5-20 amino acids, about 10-20 amino acids, about 5-15 amino acids or about 10-15 amino acids in length. In another example, the linker is about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19 or about 20 amino acids in length. Suitable linkers are known in the art and include flexible linkers such as Gly-Ser linkers.

In some examples, the peptide linker comprises the amino acid sequence of any one of SEQ ID NOs: 22 to 27.

In another example, the capsid protein is fused to the TM protein domain by a hinge.

In some examples, the capsid protein is positioned C-terminal to the antigen protein and TM protein domain. For example, the capsid protein may comprise, in N- to C-terminal order: the antigen protein, the TM protein domain and then the capsid protein. Adjacent components of the capsid fusion protein may be fused via a peptide linker.

In an example, the TM protein domain and the antigen protein are from a virus. In another example, the TM protein domain and the antigen protein are from the same virus. For example,

the TM protein domain and the antigen protein are from influenza. In another example, the TM protein domain and the antigen protein are from respiratory syncytial virus (RSV). In another example, the TM protein domain and the antigen protein are from a SARS-CoV-2.

Thus, in one example of the disclosure, there is provided a recombinant virus-like particle (VLP) comprising a capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:

(a) a capsid protein from an AMV, bacteriophage AP205 or bacteriophage MS2 non-enveloped virus;

(b) a transmembrane (TM) protein domain from a HA or NA protein of an influenza virus; and

(c) an antigen protein from a HA or NA protein of an influenza virus, wherein the capsid protein is encapsulated within the lipid bilayer.

In one example, the TM protein domain and the antigen protein are from an influenza A virus strain. For example, the TM protein domain and the antigen protein are from an influenza A virus hemagglutinin (HA) protein, a neuraminidase (NA) protein, a matrix (M) protein, a nucleoprotein (NP), a non-structural (NS) protein, or an immunogenic fragment or variant thereof. In one example, the TM protein domain and the antigen protein are from an influenza A hemagglutinin (HA) subtype H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 and/or an influenza A neuraminidase (NA) subtype N1, N2, N3, N4, N5, N6, N7, N8 or N9 and/or an influenza A matrix (M) protein subtype M1 or M2 and/or an influenza A non-structural (NS) protein subtype NS1 or NS2.

The skilled person will be aware that pandemic strains of the influenza virus are commonly H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strains. For example, H1N1, H2N2, H3N2, H5N1, H5N3, H6N1, H7N2, H7N3, H7N7, H7N9 and H9N2, strains. Thus, in one example, the TM protein domain and the antigen protein is a H1N1 protein from a A/Delaware/55/2019 virus strain. In one example, the TM protein domain and the antigen protein is a H1 protein from a A/Delaware/55/2019 virus strain. In another example the TM protein domain and the antigen protein is a H1N1, H2N2, H3N2, H5N1, H5N3, H6N1, H7N2, H7N3, H7N7, H7N9 and H9N2 protein.

In another example, the TM protein domain and the antigen protein are from an influenza B virus strain. The skilled person will be aware that influenza B viruses are not divided into subtypes but are classified into two lineages, namely, B/Yamagata and B/Victoria.

In one example, the TM protein domain and the antigen protein are from a B/Yamagata influenza B virus strain. For example, the influenza B virus strain is a B/Singapore/INFTT 16 0610/16 (By) virus strain. In another example, the TM protein domain and the antigen protein are from a B/Victoria influenza B virus strain. In one example, TM protein domain and the antigen protein are from an influenza B virus Hyam protein or a Nyam protein. For example, the TM protein domain and the antigen protein are from an influenza B virus Hyam protein. In

another example, the TM protein domain and the antigen protein are from an influenza B virus Nyam protein. In a further example, the TM protein domain and the antigen protein are from an influenza B virus Hyam and Nyam protein.

In one example, the TM protein domain and the antigen protein are from influenza B. In another example, the TM protein domain and the antigen protein are from influenza C.

In one example of the disclosure, there is provided a recombinant virus-like particle (VLP) comprising a capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:

(a) a capsid protein from an AMV, bacteriophage AP205 or bacteriophage MS2 non-enveloped virus;

(b) a transmembrane (TM) protein domain from a Spike (S) protein of a SARS-CoV-2; and

(c) an antigen protein from a Spike (S) protein of a SARS-CoV-2, wherein the capsid protein is encapsulated within the lipid bilayer.

In some examples, the capsid protein is from a bacteriophage MS2 virus, the TM protein domain is from a Spike (S) protein of a SARS-CoV-2 and the antigen protein is from a Spike (S) protein of a SARS-CoV-2.

Thus, in an example, the TM protein domain and the antigen protein are from a Spike (S) protein from a SARS-CoV-2. In another example, the antigen is from the Alpha (B.1.1.7) strain, the Beta (B.1.351) strain, the Gamma (P1) strain, the Epsilon (B.1.429) strain, the Delta (B.1.617.2) mutant, the Kappa (B.1.617.1) strain, the Wuhan (2019-nCoV/USA-WA1/2020) strain or the Omicron (B.1.1.529) strain of a SARS-CoV-2.

In another example, the S protein is a mutant S protein.

In one example, a mutant S protein comprises a mutation in the receptor binding domain. For example, the mutation is selected from the group consisting of S438F, N439K, N440K, L441I, K444R, V445A, V445I, G446V, G446S, N450K, L452R, L452P, L455F, K458N, N460T, D467V, I468F, I468T, I468V, E471O, I472V, A475V, G476S, S477G, S477I, S477N, S477R, T478I, P479L, P479R, P479S, N481D, N481H, V483F, V483A, E484D, E484K, E484Q, G485S, Y489H, Y489D, Y489F, Y489C, Y489N, F490L, F490S, P491R, Q493L, S494P, Y495N, T500N, N501S and Y505H, Y508H. In one example, a mutant S protein comprises a mutation in the receptor binding domain selected from the group consisting of N439K, N439L, L452R, S477N, T478I, V483A and E484D.

In one example, a mutant S protein comprises a mutation in the receptor binding domain. For example, the mutation is selected from the group consisting of R346K, K417N, K417T, S438F, N439K, N440K, L441I, K444R, V445A, V445I, G446V, G446S, N450K, L452R, L452P, L455F, K458N, N460T, D467V, I468F, I468T, I468V, E471O, I472V, A475V, G476S, S477G, S477I, S477N, S477R, T478I, T478K, P479L, P479S, N481D, N481H, V483F, V483A, E484D, E484K, E484Q, G485S, Y489H, Y489D, Y489F, Y489C, Y489N, F490L, F490S, P491R, Q493L, S494P, Y495N, T500N, N501S, N501Y, Y505H and Y508H. In one

example, a mutant S protein comprises a mutation in the receptor binding domain selected from the group consisting of R346K, K417N, K417T, N439K, N439L, L452R, S477N, T478I, V483A, E484D, E484K and N501Y.

In one example, a mutant S protein comprises a mutation selected from the group consisting of P337S, F338L, F338C, G339D, E340K, V341I, A344S, T345S, R346K, A348S, A348T, W353R, N354D, N354K, N354S, S359N, D364Y, V367F, S373L, V382L, P384L, P384S, T385A, T393P, V395I, F400C, R403K, R403S, D405V, R408I, Q414E, Q414K, Q414P, Q414R, T415S, K417R, K417N, I418V, Y421S, Y423C, Y423F, Y423S, D427Y, R509K, V510L, V511E, V512L, L518I, H519O, A520S, A520V, P521R, P521S, A522P, A522S and D614G.

In one example, a mutant S protein comprises a mutation selected from the group consisting of L18F, D80A, T95I, Y144S, Y145N, D215G, P337S, F338L, F338C, G339D, E340K, V341I, A344S, T345S, R346K, A348S, A348T, W353R, N354D, N354K, N354S, S359N, D364Y, V367F, S373L, V382L, P384L, P384S, T385A, T393P, V395I, F400C, R403K, R403S, D405V, R408I, Q414E, Q414K, Q414P, Q414R, T415S, K417N, K417T, K417R, I418V, Y421S, Y423C, Y423F, Y423S, D427Y, S438F, N439K, N440K, L441I, K444R, V445A, V445I, G446V, G446S, N450K, L452R, L452P, L455F, K458N, N460T, D467V, I468F, I468T, I468V, E471O, I472V, A475V, G476S, S477G, S477I, S477N, S477R, T478I, T478K, P479L, P479S, N481D, N481H, V483F, V483A, E484D, E484K, E484K, E484O, G485S, Y489H, Y489D, Y489F, Y489C, Y489N, F490L, F490S, P491R, Q493L, S494P, Y495N, T500N, N501S, N501Y, Y505H, Y508H, R509K, V510L, V511E, V512L, L518I, H519O, A520S, A520V, P521R, P521S, A522P, A522S, A570D, D614G, P680H, P681H, A701V, T716I and D950N.

In some examples, the S protein comprises a D to G mutation at the residue corresponding to position 614 of SEQ ID NO: 38 (i.e., a D614G mutation).

In one example, the S protein lacks a furin cleavage site at the the S1/S2 boundary and/or the S2' site. Thus, the amino acid sequence of the furin cleavage sites may be modified/susbstituted to inhibit furin cleavage.

In some examples, the S protein comprises RRAR to QQAA mutations at residues corresponding to positions 682-685 of SEQ ID NO: 38.

In some examples, the S protein comprises RRAR to GSAS mutations at residues corresponding to positions 682-685 of SEQ ID NO: 38.

In some examples, comprises the S protein comprises insertion of two proline residues between residues corresponding to positions 986 and 987 of SEQ ID NO: 38.

In some examples, the S protein (i) lacks a furin cleavage site at the S1/S2 boundary; and/or (ii) comprises RRAR to QQAA mutations or RRAR to GSAS mutations at residues corresponding to positions 682-685 of SEQ ID NO: 38; and/or (iii) lacks a furin cleavage site at the S2' site; and/or (iv) comprises a D to G mutation at the residue corresponding to position 614

of SEQ ID NO: 38; and/or (v) comprises insertion of two proline residues between residues corresponding to positions 986 and 987 of SEQ ID NO: 38.

In some examples, the capsid fusion protein comprises an S protein, comprising the TM protein domain and antigen protein, wherein the S protein comprises an amino acid sequence selected from any one of SEQ ID NOs: 29, 31 or 38, or an amino acid sequence which is at least 90% identical to any one of SEQ ID NOs: 29, 31 or 38.

In some examples, the capsid fusion protein comprises an S protein, comprising the TM protein domain and antigen protein, wherein the S protein comprises the amino acid sequence of SEQ ID NO: 29, or an amino acid sequence at least 90% identical to SEQ ID NO: 29.

In some examples, the capsid fusion protein comprises an S protein, comprising the TM protein domain and antigen protein, wherein the S protein comprises the amino acid sequence of SEQ ID NO: 31, or an amino acid sequence at least 90% identical to SEQ ID NO: 31.

In some examples, the capsid fusion protein comprises an S protein, comprising the TM protein domain and antigen protein, wherein the S protein comprises the amino acid sequence of SEQ ID NO: 38, or an amino acid sequence at least 90% identical to SEQ ID NO: 38.

In some examples, the capsid fusion protein comprises an S protein, comprising the antigen protein, wherein the S protein comprises the amino acid sequence of SEQ ID NO: 30, or an amino acid sequence at least 90% identical to SEQ ID NO: 30. SEQ ID NO: 30 is the amino acid sequence of the SARS-CoV-2 Spike protein truncated at the C-terminus to remove the TM protein domain.

In one example of the disclosure, there is provided a recombinant virus-like particle (VLP) comprising a capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:

(a) a capsid protein from an AMV, bacteriophage AP205 or bacteriophage MS2 non-enveloped virus;

(b) a transmembrane (TM) protein domain from a pre F or F protein of a respiratory syncytial virus (RSV); and

(c) an antigen protein from a pre F or F protein of a RSV,
wherein the capsid protein is encapsulated within the lipid bilayer.

Thus, in one example, the TM protein domain and the antigen protein are from a respiratory syncytial virus (RSV). For example, the TM protein domain and the antigen protein are selected from a RSV surface glycoprotein including a Fusion (F), Glycoprotein (G), Small Hydrophobic protein (SH), the matrix proteins M and M2, the nucleocapsid proteins N, P and L, and the nonstructural proteins NS1 and NS2.

In one example, the TM protein domain and the antigen protein are from a Pre F protein of a RSV.

In another example, the TM protein domain and the antigen protein are from different viruses.

For example, the TM protein domain is from a S protein from a SARS-CoV-2 (i.e., the TM protein domain thereof) and the antigen protein is from an influenza A virus HA protein or NA protein. In another example, the TM protein domain is an influenza A virus HA protein or NA protein (i.e., the TM protein domain thereof) and the antigen protein is a S protein from a SARS-CoV-2. In an embodiment, the S protein, the HA protein or NA protein are selected from any of those described herein or known in the art.

In another example, the TM protein domain is a S protein from a SARS-CoV-2 (i.e., the TM protein domain thereof) and the antigen protein is a F or Pre F protein of a RSV. In another example, the TM protein domain is a F or Pre F of a RSV (i.e., the TM protein domain thereof) and the antigen protein is a S protein from a SARS-CoV-2. In an embodiment, the S protein, the HA protein or NA protein are selected from any of those described herein or known in the art.

In another example, the TM protein domain is a F or Pre F of a RSV (i.e., the TM protein domain thereof) and the antigen protein is an influenza A virus HA protein or NA protein. In another example, the TM protein domain is an influenza A virus HA or NA TM protein domain (i.e., the TM protein domain thereof) and the antigen protein is a F or Pre F protein of a RSV. In an embodiment, the S protein, the HA protein or NA protein are selected from any of those described herein or known in the art.

In some examples, the capsid fusion protein comprises the amino acid sequence of any one of SEQ ID NOs: 1 to 20, or an amino acid sequence at least 90% identical to any one of SEQ ID NOs: 1 to 20. The polyhistidine tag GSSH HHHHH (SEQ ID NO: 28) may or may not be present in the capsid fusion protein sequences.

In some examples, the capsid fusion protein comprises the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence at least 90% identical to SEQ ID NO: 5 (optionally lacking the polyhistidine tag GSSH HHHHH).

In some examples, the capsid fusion protein comprises the amino acid sequence of SEQ ID NO: 12, or an amino acid sequence at least 90% identical to SEQ ID NO: 12 (optionally lacking the polyhistidine tag GSSH HHHHH).

In some examples, the capsid fusion protein comprises the amino acid sequence of SEQ ID NO: 15, or an amino acid sequence at least 90% identical to SEQ ID NO: 15 (optionally lacking the polyhistidine tag GSSH HHHHH).

In some examples, the capsid fusion protein comprises the amino acid sequence of SEQ ID NO: 17, or an amino acid sequence at least 90% identical to SEQ ID NO: 17 (optionally lacking the polyhistidine tag GSSH HHHHH).

In some examples, the capsid fusion protein comprises the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence at least 90% identical to SEQ ID NO: 18 (optionally lacking the polyhistidine tag GSSH HHHHH).

In some examples, the capsid fusion protein comprises the amino acid sequence of SEQ ID NO: 20, or an amino acid sequence at least 90% identical to SEQ ID NO: 20 (optionally lacking the polyhistidine tag GSSHHHHHH).

In one example, the VLP comprises a second capsid fusion protein. In one embodiment, the second capsid fusion protein comprises an antigen protein that is different to the first antigen protein. In this embodiment, the TM protein domain of the second capsid fusion protein may be the same to the TM protein domain of the first capsid fusion protein. In another embodiment, the second capsid fusion protein comprises a TM protein domain that is different to the first TM protein domain of the first capsid fusion protein.

In an example, where a second capsid fusion protein is contemplated, each of the antigens are formulated in separate VLPs or the same VLP. For example, a capsid fusion protein comprising the S protein and a capsid fusion protein comprising the F or Pre F protein may be formulated in the same VLP. In another example, a capsid fusion protein comprising the S protein and a capsid fusion protein comprising the F or Pre F protein may be formulated in different VLPs. In another example, a capsid fusion protein comprising the HA or NA protein and a capsid fusion protein comprising the F or Pre F protein may be formulated in the same VLP. In another example, a capsid fusion protein comprising the HA or NA protein and a capsid fusion protein comprising the F or Pre F protein may be formulated in different VLPs. In another example, a capsid fusion protein comprising the HA or NA protein and a capsid fusion protein comprising the S protein may be formulated in the same VLP. In another example, a capsid fusion protein comprising the HA or NA protein and a capsid fusion protein comprising the S protein may be formulated in different VLPs.

In an example, the VLP does not include any viral RNA. In other words, the VLP lacks RNA that is capable of infecting and replicating in a host. Thus, the VLPs of the present disclosure are considered to be non-infective. In another example, the VLP is substantially free of viral RNA or has less than about 10%, 5%, 1%, or 0.1%, preferably less than about 5%, more preferably less than about 1% viral RNA by weight.

In an example, the VLP has a diameter of between about 70nm and 160nm, between about 70nm and 150nm, between about 70nm and 140nm, between about 70nm and 130nm, between about 70nm and 120nm, between about 70nm and 110nm, between about 70nm and 100nm, or between about 70nm and 90nm. In another example, the VLP has a diameter of about 80nm.

In an example, the VLP has a diameter of between about 30nm and 120nm, between about 40nm and 110nm, between about 50nm and 100nm, between about 60nm and 90nm or between about 70nm and 80nm.

In one example, the VLP is formulated in a lipid nanoparticle (LNP). For example, the VLP is encapsulated in a LNP. In another example, the VLP is bound to the LNP. In another example, the VLP is absorbed on the LNP.

In one example, the LNP further comprises a PEG-lipid, a structural lipid and/or a neutral lipid. For example, the LNP further comprises a PEG-lipid. In another example, the LNP further comprises a structural lipid. In another example, the LNP further comprises a neutral lipid.

In one example, the LNP comprises an ionisable lipid. For example, the ionisable lipid is a cationic lipid. In another example, the ionisable lipid is a zwitterionic lipid.

In one example, the LNP does not comprise an ionisable lipid.

In one example, where multiple VLPs are contemplated, each VLP is formulated together in a LNP. For example, a capsid fusion protein comprising the S protein and a capsid fusion protein comprising the F or Pre F protein may be formulated in separate VLPs and then formulated together in the LNP. In another example, a capsid fusion protein comprising the HA or NA protein and a capsid fusion protein comprising the F or Pre F protein may be formulated in separate VLPs and then formulated together in the LNP. In another example, a capsid fusion protein comprising the S protein and a capsid fusion protein comprising the HA or NA protein may be formulated in separate VLPs and then formulated together in the LNP.

In one example, each VLP is formulated separately in the LNP. For example, For example, a capsid fusion protein comprising the S protein and a capsid fusion protein comprising the F or Pre F protein may be formulated in separate VLPs and then formulated separately in the LNP. In another example, a capsid fusion protein comprising the HA or NA protein and a capsid fusion protein comprising the F or Pre F protein may be formulated in separate VLPs and then formulated separately in the LNP. In another example, a capsid fusion protein comprising the S protein and a capsid fusion protein comprising the HA or NA protein may be formulated in separate VLPs and then formulated separately in the LNP.

The present disclosure also provides a capsid fusion protein comprising:

- (a) a capsid protein from a non-enveloped virus;
- (b) a transmembrane (TM) protein domain; and
- (c) an antigen protein.

The present disclosure also provides a capsid fusion protein comprising:

- (a) a capsid protein from a non-enveloped virus; and
- (b) an antigen protein.

Herein, examples of features of the capsid fusion proteins in the VLPs of the disclosure apply mutatis mutandis to the capsid fusion proteins of the disclosure.

In one example, the present disclosure further provides an isolated, recombinant or synthetic nucleotide sequence encoding a capsid fusion protein disclosed herein. In another example, the present disclosure further provides an isolated, recombinant or synthetic nucleotide sequence encoding a VLP disclosed herein.

In one example, the isolated, recombinant or synthetic nucleotide sequence encodes a capsid fusion protein, the nucleotide sequence comprising in 5' to 3' order:

(a) a polynucleotide encoding a capsid protein from an AMV, bacteriophage AP205 or bacteriophage MS2 non-enveloped virus;

(b) a polynucleotide encoding a transmembrane (TM) protein domain from a Spike (S) protein of a SARS-CoV-2; and

(c) a polynucleotide encoding an antigen protein from a Spike (S) protein of a SARS-CoV-2,

wherein the polynucleotides are operably linked to a regulatory element.

In an example, the regulatory element is a promoter. In one example, the promoter is a synthetic genomic promoter.

In one example, the isolated, recombinant or synthetic nucleotide sequence further encodes a signal peptide 5' to the polynucleotide encoding the antigen. In another example, the polynucleotides encoding the TM protein and the antigen protein are operably linked by a polynucleotide encoding a linker.

In one example, the present disclosure further provides an expression vector comprising a nucleotide sequence that encodes a capsid fusion protein disclosed herein. In another example, the present disclosure further provides an expression vector comprising a nucleotide sequence that encodes a VLP disclosed herein.

In one example, the present disclosure further provides a pharmaceutical composition comprising a VLP disclosed herein and a pharmaceutically acceptable carrier. In one example, the pharmaceutical composition is an immunogenic composition.

In another example, the present disclosure further provides a pharmaceutical composition comprising a VLP disclosed herein for use as a vaccine. In another example, present disclosure further provides a vaccine comprising the pharmaceutical or immunogenic composition.

In an example, the composition further comprises an adjuvant. In an example, the adjuvant is selected from the group consisting of Freund's adjuvant, incomplete Freund's adjuvants, aluminum phosphate, aluminum hydroxide, GMCSF, BCG, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, monophosphoryl lipid A (MPL), RIBI, MPL, trehalose dimycolate (TDM), Novasomes®, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide, MHC antigens, PolyI:C, MF59, glycolipid analogs, octadecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, ODA, cytokines, and other adjuvants and derivatives thereof. In an example, the adjuvant is MF59.

In an example, an adjuvant such as MF59 is administered at the same time as the administration of a composition of the disclosure. In another example, an adjuvant such as MF59 is administered sequentially to, preceding, or proceeding the administration of a composition of the disclosure.

In an example, the present disclosure provides a method of treating or preventing or delaying progression of a disease, disorder or condition in a subject in need thereof, the method

comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for treating or preventing or delaying progression of a disease, disorder or condition in a subject.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in the treatment or prevention or delaying progression of a disease, disorder or condition in a subject.

In an example, the disease, disorder or condition is a viral infection.

In an example, the viral infection is COVID-19, influenza or RSV.

In an example, the subject having a viral infection has at least one symptom of COVID-19, influenza or RSV. In an example, one such symptom includes runny nose, cough, sore throat, fever, headache, muscle pain or fatigue.

In an example, the present disclosure provides a method of inducing an immune response in a subject, the method comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in inducing an immune response in a subject in need thereof.

In one example, the composition induces a humoral immune response in the subject. For example, the humoral immune response is an antibody-mediated immune response. For example, production of neutralizing antibodies. In another example, the composition induces a cell-mediated immune response. For example, the cell-mediated immune response includes activation of antigen-specific cytotoxic T cells. For example, the T cells are CD4 T cells and/or CD8 T cells. In one example, the T cells are CD4 T cells. In another example the T cells are CD8 T cells. In a further example, the T cells are CD4 and CD8 T cells.

In one example, administration of a VLP, a pharmaceutical composition, an immunogenic composition or a vaccine of the present disclosure induces a CD4 T cell mediated immune response.

In one example, administration of a VLP, a pharmaceutical composition, an immunogenic composition or a vaccine of the present disclosure induces a CD8 T cell mediated immune response.

In one example, administration of a VLP, the pharmaceutical composition, an immunogenic composition or a vaccine of the present disclosure induces a CD4 and a CD8 T cell mediated immune response.

In one example, the CD4 T cell mediated immune response is a Th0, a Th1 and/or a Th2 response. For example, the CD4 T cell mediated immune response is a Th0 response. In another example, the CD4 T cell mediated immune response is a Th1 response. In a further example, the CD4 T cell mediated immune response is a Th2 response. In one example, the CD4 T cell mediated immune response is a Th0 and Th1 response. In another example, the CD4 T cell mediated immune response is a Th0 and Th2 response. In a further example, the CD4 T cell mediated immune response is a Th1 and Th2 response. In another example, the CD4 T cell mediated immune response is a Th0, Th1 and Th2 response.

In one example, the Th0 response cytokines express interleukin 2 (IL2+) and/or tumor necrosis factor alpha (TNFα+); and/or are negative for interferon gamma (IFNγ-), IL5- and/or IL13-. For example, the cytokine is IL2+. In another example, the cytokine is TNFα+. In one example, the cytokine is IFNγ-. In another example, the cytokine is IL5-. In a further example, the cytokine is IL13-.

In one example, the Th1 response cytokines express interferon gamma (IFNγ+); and/or are negative for IL5- and/or IL13-. For example, the cytokine is IFNγ+. In another example, the cytokine is IL5-. In a further example, the cytokine is IL13-.

In one example, the Th2 response cytokines express IL5+ and/or IL13+; and/or are negative for IFNγ. For example, the cytokine is IL5+. In a further example, the cytokine is IL13+. For example, the cytokine is IFNγ-.

In one example, the immune response is raised in response to at least one antigen from a SARS-CoV-2, influenza or RSV. For example, the immune response is raised in response to an S protein antigen from a SARS-CoV-2 described herein. In one example, the immune response is raised in response an influenza antigen described herein such as a HA or NA protein antigen. In another example, the immune response is raised in response a RSV antigen described herein such as a F protein or Pre F protein.

In another example, the immune response is sufficient to treat, prevent or delay progression of at least one symptom of a viral infection caused by a SARS-CoV-2, influenza or RSV.

In an example, the present disclosure provides a method for reducing viral load in a subject comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for reducing viral load in a subject.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in reducing viral load in a subject.

In an example, the present disclosure provides a method for treating, preventing or delaying progression of pneumonia comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for treating, preventing or delaying progression of pneumonia in a subject.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in treating, preventing or delaying progression of pneumonia in a subject.

In an example, the present disclosure provides a method for treating, preventing or delaying progression of acute respiratory distress syndrome in a subject comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for treating, preventing or delaying progression of acute respiratory distress syndrome in a subject.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in treating, preventing or delaying progression of acute respiratory distress syndrome in a subject.

In an example, the subject is a human of 18 years of age or older. In another example, the subject is a human of any age, e.g., from about 1 month to 100 years old, e.g., from about 2 months to about 80 years old, from about 6 months of age to about 3 years old, from about 3 years to about 18 years old, from about 12 years to about 18 years old, from about 18 years to about 55 years old, from about 50 years to about 75 years old, from about 40 years to about 65 years old. In another example, the subject is a human from 2 years of age. In another example, subject is a human from 18 years of age, a human from 30 years of age, a human from 40 years of age, a human from 50 years of age, a human from 60 years of age, a human from 70 years of age, a human from 80 years of age or a human from about 90 years of age. In another example,

the subject is less than 2 years of age, less than 18 months of age, less than 12 months of age, less than 6 months of age or less than 3 months of age.

In an example, a composition or vaccine described herein is administered in a one dose regimen. In another example, the composition is administered in a two, three or four dose regimen. In this example, the doses may be administered about 1, 2 or 3 months apart.

In an example of the present disclosure, there is provided a eukaryotic cell for expressing a VLP described herein. In an example, the eukaryotic cell is a CHO cell, baby hamster kidney-21 (BHK-21) cell, human embryonic kidney 293 (HEK293) cell, CAP-T cell line derived from human amniocytes, Vero 9, or an east lansing line-0 (ELL-0) cell.

In an example, the eukaryotic cell comprises polynucleotides encoding a capsid fusion protein, the capsid fusion protein comprising:

- (a) a capsid protein from a non-enveloped virus;
- (b) a transmembrane (TM) protein domain; and
- (c) an antigen protein.

In an example of the present disclosure, there is provided a method for producing a VLP comprising:

- (a) providing one or more expression vectors comprising polynucleotides for expression of the viral like particle (VLP) described herein,
 - (b) providing a host cell, and
 - (c) transfecting said host cell with said one or more expression vectors to produce virus-like particles (VLPs) comprising the one or more antigens,
- wherein the polynucleotides are expressed under conditions sufficient for the formation of VLPs.

In an example of the present disclosure, there is provided a method for producing a VLP comprising:

- (a) providing an expression vector comprising polynucleotides encoding a capsid fusion protein, the capsid fusion protein comprising:
 - (i) a capsid protein from a non-enveloped virus;
 - (ii) a transmembrane (TM) protein domain; and
 - (iii) an antigen protein,
 - (b) providing a host cell, and
 - (c) transfecting said host cell with said vector to produce virus-like particles (VLPs) comprising the one or more antigens,
- wherein the polynucleotides are expressed under conditions sufficient for the formation of VLPs, wherein the VLP, once formed, comprises a lipid bilayer; and wherein the capsid protein is encapsulated within the lipid bilayer.

In an example, the method further comprises purifying the VLPs.

In an example, the present disclosure also provides a kit comprising at least one composition or vaccine of the disclosure.

In one example, the kit comprises a composition or vaccine of the present disclosure, optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions for use in treating or preventing or delaying progression of a viral infection in a subject in need thereof. In an example, the composition or vaccine comprises an adjuvant such as MF59. In another example, the kit further comprises a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions to administer the VLP to a subject who is suffering from or at risk of suffering from a viral infection.

Thus, in one example, the kit comprises:

- (a) a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein;
- (b) instructions for use thereof; and optionally
- (c) a pharmaceutically acceptable carrier, excipient or diluent.

In one example, the composition, the immunogenic composition or the pharmaceutical composition of the disclosure is supplied in a vial. In another example, the immunogenic composition or the pharmaceutical composition of the disclosure is supplied in a syringe.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

BRIEF DESCRIPTION OF FIGURES

Figure 1. Schematic of an exemplary capsid fusion protein comprising a capsid protein, linker, transmembrane domain and antigen protein.

Figure 2. Exemplary VLP constructs of the disclosure described in Example 1. **A)** Exemplary VLP that includes a AMV capsid protein. **B)** Exemplary VLP that includes bacteriophage capsid protein AP205. **C)** Exemplary VLP that includes bacteriophage capsid protein MS2.

Figure 3. Exemplary VLP constructs of the disclosure that include an AMV capsid protein.

Figure 4. Exemplary VLP constructs of the disclosure that include a bacteriophage AP205 protein.

Figure 5. Exemplary VLP constructs of the disclosure that include a bacteriophage MS2 protein.

Figure 6. Electron micrograph of VLPs comprising the MS006 construct. Successfully formed VLPs are indicated by grey boxes.

DETAILED DESCRIPTION

General

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter.

Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present disclosure.

Any example of the present disclosure herein shall be taken to apply *mutatis mutandis* to any other example of the disclosure unless specifically stated otherwise. Stated another way, any specific example of the present disclosure can be combined with any other specific example of the disclosure (except where mutually exclusive).

Any example of the present disclosure disclosing a specific feature or group of features or method or method steps will be taken to provide explicit support for disclaiming the specific feature or group of features or method or method steps.

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J. Sambrook *et al.* Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan *et al.* (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

Throughout this specification the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein the term “derived from” shall be taken to indicate that a specified integer can be obtained from a particular source albeit not necessarily directly from that source. Similarly, the term “based on” shall be taken to indicate that a specified integer can be developed or used from a particular source albeit not necessarily directly from that source.

Selected Definitions

As used herein, the term “fragment” refers to a portion of a nucleotide sequence or polypeptide (protein) of a reference nucleotide sequence or polypeptide disclosed herein which maintains a defined activity of the full length nucleotide sequence or polypeptide. In one example, the defined activity is inducing an immune response in a subject administered with a composition of the present disclosure.

As used herein, the term “variant” refers to a nucleotide sequence or polypeptide (e.g. antigenic polypeptide) with difference(s) in one or more nucleotide sequence(s) or amino acid sequence(s) to a reference nucleotide sequence or polypeptide disclosed herein which maintains a defined activity of the nucleotide sequence or polypeptide. The difference(s) in one or more nucleotide sequence(s) or amino acid sequence(s) results from one or modification(s) made to the nucleotide sequence or polypeptide of the present disclosure. In one example, the modification is a chemical modification of one or more nucleotide(s) of the nucleotide sequence. For example, at least one naturally occurring nucleotide of the RNA is replaced with a chemically modified nucleotide (e.g. pseudouridine (ψ), and 1-methylpseudouridine (m1 ψ)). In one example, the modification comprises increasing the G/C content of the nucleotide sequence. In one example, the modification comprises codon optimization of the nucleotide sequence. In one example, the defined activity is inducing an immune response in a subject administered with a composition of the present disclosure.

In one example, the variant has at least 70%, or 75%, or 80%, or 85%, or 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% of sequence identity with a sequence disclosed herein. In one example, the variant has at least 70%, or 75%, or 80%, or 85%, or 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% of sequence identity with a sequence disclosed herein. The nucleotide sequence or polypeptide variant disclosed herein can have one or more nucleotide(s) or amino acid(s) deleted or substituted by different nucleotide(s) or amino acid(s). In one example, the substitution is a conservative substitution. A skilled person will appreciate that a conservative substitution with reference to a

polypeptide involves replacement of an amino acid in the polypeptide with a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity and size). In one example, the substitution is a non-conservative substitution.

As used herein, the term “encode”, “encodes” or “encoding” refers to a region of a RNA capable of undergoing translation into a polypeptide.

As used herein, the term “antigen” refers to a molecule or structure containing one or more epitopes that induce, elicit, augment or boost a cellular and/or humoral immune response. Antigens can include, for example, proteins and peptides from a pathogen such as a virus, bacteria, fungus, protozoan, plant or from a tumour. For example, an antigen is derived from a gene of interest. In another example, the antigenic proteins are from a SARS-CoV-2, RSV or influenza. In another example, the antigenic proteins are from an S protein of a SARS-CoV-2, an F or Pre F protein of a RSV or a HA or NA protein from influenza.

As used herein the term "adjuvant" refers to a compound that, when used in combination with a specific immunogen (e.g. a VLP) in a formulation, augments or otherwise alters or modifies the resultant immune response. Modification of the immune response includes intensification or broadening the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses.

The term “naked” as used herein refers to nucleic acids that are substantially free of other macromolecules, such as lipids, polymers and proteins. A “naked” nucleic acid is not formulated with other macromolecules to improve cellular uptake. Accordingly, a naked nucleic acid is not encapsulated in, absorbed on, or bound to a lipid nanoparticle (LNP), a liposome or a polymeric microparticle.

As used herein, the term “nucleotide sequence” or “nucleic acid sequence” will be understood to mean a series of contiguous nucleotides (or bases) covalently linked to a phosphodiester backbone. By convention, sequences are presented from the 5' end to the 3' end, unless otherwise specified.

As used herein, the term “operably linked to” means positioning a translation initiation sequence (e.g. a Kozak consensus sequence, an internal ribosome entry site (IRES), a subgenomic (SG) promoter) or a stability element (e.g., an 5'-UTR) relative to a nucleic acid such that expression of the nucleic acid is controlled or regulated by the sequence or element. For example, a translation initiation sequence can be operably linked to the 5' end of the one or more polynucleotide sequence(s) disclosed herein.

The term “polypeptide” or “polypeptide chain” will be understood to mean a series of contiguous amino acids linked by peptide bonds. For example, a protein shall be taken to include a single polypeptide chain i.e., a series of contiguous amino acids linked by peptide bonds or a series of polypeptide chains covalently or non-covalently linked to one another (i.e., a polypeptide complex). The series of polypeptide chains can be covalently linked using a suitable

chemical or a disulfide bond. Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and hydrophobic interactions.

The term “recombinant” shall be understood to mean the product of artificial genetic recombination.

As used herein, the term “lipid nanoparticle” or “LNP” shall be understood to refer to any lipid composition, including, but not limited to, liposomes or vesicles, where an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar), micelle-like lipid nanoparticles having a non-aqueous core and solid lipid nanoparticles.

As used herein, the terms “disease”, “disorder” or “condition” refers to a disruption of or interference with normal function.

As used herein, a subject “at risk” of developing an infection e.g., a viral infection may have or may not have detectable disease or symptoms of the infection, and may have or may not have displayed detectable disease or symptoms of the infection prior to the treatment according to the present disclosure. “At risk” denotes that a subject has one or more risk factors, which are measurable parameters that correlate with development of the infection, as known in the art and/or described herein.

As used herein, the terms “treatment” or “treating” a subject includes the application or administration of a compound or composition of the disclosure to a subject (or application or administration of a compound of the disclosure to a cell or tissue from a subject) with the purpose of delaying, slowing, stabilizing, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term “treating” refers to any indication of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms or making the injury, pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating.

As used herein, “preventing” or “prevention” is intended to refer to at least the reduction of likelihood of the risk of (or susceptibility to) acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease). Biological and physiological parameters for identifying such patients are provided herein and are also well known by physicians.

As used herein, the phrase “delaying progression of” includes reducing or slowing down the progression of the disease or condition in an individual and/or at least one symptom of a disease or condition.

An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired result. For example, the desired result can be a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. In some examples of the present disclosure, the term “effective amount” is meant an amount necessary to effect treatment of a disease or condition as hereinbefore described. In some examples of the present disclosure, the term “effective amount” is meant an amount necessary to effect a change associated with a disease or condition as hereinbefore described. The effective amount can vary according to the disease or condition to be treated or factor to be altered and also according to the weight, age, racial background, sex, health and/or physical condition and other factors relevant to the mammal being treated. Typically, the effective amount will fall within a relatively broad range (e.g. a “dosage” range) that can be determined through routine trial and experimentation by a medical practitioner. Accordingly, this term is not to be construed to limit the disclosure to a specific quantity. The effective amount can be administered in a single dose or in a dose repeated once or several times over a treatment period.

A “therapeutically effective amount” refers to the minimum concentration required to effect a measurable improvement of a particular disease or condition. A therapeutically effective amount herein can vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the VLP of the present disclosure to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the VLP are outweighed by the therapeutically beneficial effects.

As used herein, the term “prophylactically effective amount” shall be taken to mean a sufficient quantity of a VLP of the disclosure to prevent or inhibit or delay the onset of one or more detectable symptoms of a disease or disorder.

A “subject” can be an animal that is susceptible to an infection within the scope of the present disclosure. A subject of this disclosure can be a mammal and in particular embodiments is a human, which can be an infant, a child, an adult or an elderly adult. A “subject at risk of infection” is any subject who may be or has been exposed to an infection such as a SARS-CoV-2, influenza or a RSV. The subject may be a primary contact of an individual diagnosed with a the relevant infection. “Subject” includes any human or non-human animal. Thus, in addition to being useful for human treatment, the compounds of the present disclosure may also be useful for veterinary treatment of mammals, including companion animals and farm animals, such as, but not limited to dogs, cats, horses, cows, sheep, and pigs.

As used herein, the term “virus-like particle”, “VLP”, “virus-like particles” or “VLPs” shall be taken to mean a multi-subunit protein- and lipid-based structure, made up elements required to produce a virus-like particle (VLP) which resembles the form and/or size of a virus particle but does not contain the genetic material of the virus. The VLP or VLPs display antigens which present conformational epitopes that elicit T cell and/or B cell immune responses but are

unable to replicate and/or infect a host cell. For example, a VLP of the present disclosure comprises one or more antigens described herein that are suitable for use as a vaccine.

Virus-like particles

Virus-like particles (VLPs) are particles which resemble viruses but do not contain viral nucleic acid and are therefore non-infectious. They commonly contain one or more virus capsid or envelope proteins which are capable of self-assembly to form the VLP. VLPs have been produced from components of a wide variety of virus families (Noad and Roy (2003), *Trends in Microbiology*, 11:438-444; Grgacic et al., (2006), *Methods*, 40:60-65). Some VLPs have been approved as therapeutic vaccines, for example Engerix-B (for hepatitis B), Cervarix and Gardasil (for human papilloma viruses).

When capsid fusion proteins of different types are contemplated, the capsid fusion proteins may be comprised in a single VLP or a number of VLPs. The skilled person will understand that VLPs can be synthesized through the individual expression of viral structural proteins, which can then self-assemble into the virus-like structure. Combinations of structural capsid proteins from different viruses can be used to create recombinant VLPs. In addition, antigens or immunogenic fragments thereof can be fused to the surface of VLPs. By way of a non-limiting example, antigens or immunogenic fragments thereof of the disclosure may be coupled to a VLP using the SpyCatcher-SpyTag system (as described by Brune, Biswas, Howarth).

The capsid fusion protein may further comprise a signal sequence to target the fusion protein to a particular site in a host cell (e.g., ER, chloroplast) or to direct extracellular secretion of the fusion protein. The absence of a signal peptide results in translation of the proteins in the cytoplasm. Any signal sequence appropriate for the host cell may be utilised, or the signal sequence may be omitted. Signal sequences have been found to be conserved across phyla and kingdoms, and, in general, almost any signal sequence may be used. Bennett and Scheller, *PNAS* 90: 2559-2563, 1993; Lührink and Sinning, *Biochim. Biophys. Acta* 1694: 17-35, 2005; Doudna and Batey, *Ann. Rev. Biochem.* 73: 539-557, 2004; Stern, et al., *Trends in Cell and Mol. Biol.* 2: 1-17, 2007.

In one example, a fusion protein of the disclosure comprises an antigen peptide of the disclosure as well as a TM peptide domain and one or more of: Hepatitis B surface antigen (HBsAg); human papillomavirus (HPV) 18 L1 protein; HPV 16 L1 protein; and/or Hepatitis E P239, preferably Hepatitis B surface antigen. In one example, said one or more fusion protein may take the form of a VLP. Without being bound by theory, this is because HBsAg, HPV 18 L1 protein, HPV 16 L1 protein and Hepatitis E P239 protein are known to spontaneously form VLPs when expressed recombinantly, and this structure is retained when HBsAg, HPV 18 L1 protein, HPV 16 L1 protein and/or Hepatitis E P239 protein are present in fusion protein form combined with an antigen and TM domain of the disclosure.

Capsid fusion proteins

The present disclosure provides for recombinant virus-like particles (VLPs) comprising a capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:

- (a) a capsid protein from a non-enveloped virus;
- (b) a transmembrane (TM) protein domain; and
- (c) an antigen protein,

wherein the capsid protein is encapsulated within the lipid bilayer.

A skilled person will understand that the capsid fusion proteins for use in present disclosure are capable of self-assembly into recombinant VLPs during expression in a suitable cell such as a CHO or HEK-293 cell. During self assembly, the lipid bilayer forms from the host cell which encapsulates the capsid protein. In this aspect of the disclosure, the VLP provides for a more controlled immunogenic response whereby an immune response is elicited against the antigen and not the capsid protein of the capsid fusion protein.

In one embodiment, the capsid fusion protein comprises a capsid protein from Alfalfa Mosaic Virus (AMV), bacteriophage AP205 or bacteriophage MS2, bound to a transmembrane domain and antigen by a linker according to the schematic provided in Figure 1. In one example, the antigen and the TM protein domain are from the same virus. For example, the antigen and the TM protein domain are from the S protein of a SARS-CoV-2. In another example, the antigen and the TM protein domain are from the F or Pre F protein of a RSV. In yet another example, the antigen and the TM protein domain are from a HA or NA protein of influenza. In one example, the antigen and the TM protein domain are from different viruses. In this embodiment, it is envisaged that the immunogenic response is elicited as a result of expression of the antigen. In one example, the antigen is from the S protein of a SARS-CoV-2 and the TM protein domain is from a F or Pre F protein of a RSV. In another example, the antigen is from the HA or NA protein of influenza and the TM protein domain is from a RSV. In yet another example, the antigen is from the S protein of a SARS-CoV-2 and the TM protein domain is from the HA or NA protein of influenza.

In another example, particularly useful but non limiting capsid fusion proteins for use in the present disclosure are exemplified in Figure 2, and include:

1. AMV004 which comprises a capsid protein from Alfalfa Mosaic Virus (AMV), a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide;
2. AP003 which comprises a capsid protein from bacteriophage AP205, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide;
3. AP007 which comprises a capsid protein dimer from bacteriophage AP205, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide;

4. MS003 which comprises a capsid protein from bacteriophage MS2, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide; and

5. MS007 which comprises a capsid protein dimer from bacteriophage MS2, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide.

In any of the capsid fusion proteins listed above, the TM domain and antigen may be linked to the capsid protein by a linker amino acid sequence.

Capsid protein

A capsid protein for use in the capsid fusion proteins of the present disclosure may be any suitable capsid protein from a non-enveloped virus, as understood by a skilled person in the art. Non enveloped viruses include norovirus, enterovirus, adenovirus and rhinovirus. A non-enveloped virus is understood in the art to not include a lipid membrane, however during expression of a VLP in a suitable cell according to the present disclosure, a lipid bilayer membrane forms from the host cell in which the VLP is expressed.. Suitable capsid proteins from non-enveloped viruses include an Alfalfa Mosaic Virus (AMV), bacteriophage AP205 or bacteriophage MS2.

The capsid protein of an AMV is an example of a non-enveloped virus suitable for use in the present disclosure and is a porous particle that provides for efficient conjugation of antigen to the N-terminus.

The capsid protein of an bacteriophage AP205 is another non-enveloped virus suitable for use in the present disclosure comprising N and C terminals close together which are exposed. This structural configuration provides for ease of antigen attachment.

The capsid protein of bacteriophage MS2 is another non-enveloped virus suitable for use in the present disclosure comprising N and C terminals close together which are exposed. The threefold symmetry is ideal for trimeric antigen attachment.

Transmembrane (TM) protein domain

In one embodiment, the capsid fusion proteins of the present disclosure comprise a transmembrane domain. The term “TM domain” and “transmembrane domain” and “transmembrane protein domain” are used interchangeably and refer to a protein sequence that spans the lipid bilayer of the VLP. The TM protein domain may be derived from any protein including any of those described herein or known in the art. Methods for determining the TM of a protein are known in the art (Elofsson et al. (2007) Annu. Rev. Biochem. 76:125-140; Bemsel et al. (2005) Protein Science 14:1723-1728).

The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein.

In one example, the TM protein domain is a S protein TM protein domain from a SARS-CoV-2 and the antigen protein is an influenza A virus HA protein or NA protein. In another example, the TM protein domain is an influenza A virus HA TM domain protein or NA TM domain protein and the antigen protein is a S protein from a SARS-CoV-2. In an embodiment, the S protein, the HA protein or NA protein are selected from any of those described herein or known in the art.

In another example, the TM protein domain is a S protein TM protein domain from a SARS-CoV-2 and the antigen protein is a F or Pre F protein of a RSV. In another example, the TM protein domain is a F or Pre F TM protein domain of a RSV and the antigen protein is a S protein from a SARS-CoV-2. In an embodiment, the S protein, the F or Pre F protein are selected from any of those described herein or known in the art.

In another example, the TM protein domain is a F or Pre F TM protein domain of a RSV and the antigen protein is an influenza A virus HA protein or NA protein. In another example, the TM protein domain is an influenza A virus HA TM protein domain or NA TM protein domain and the antigen protein is a F or Pre F protein of a RSV. In an embodiment, the F or Pre F protein or the F or Pre F protein are selected from any of those described herein or known in the art.

The TM domain may include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid(s) associated with the extracellular region of the protein from which the transmembrane was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region).

In some instances, the transmembrane domain can be attached to the extracellular region of the chimeric polypeptide via a hinge, e.g., a hinge from a human protein. For example, in one embodiment, the hinge can be a human Ig (immunoglobulin) hinge, e.g., an IgG4 hinge, or a CD8 α hinge.

A skilled person will understand that a hinge region, as distinguished from a spacer or linker region, is a flexible amino acid stretch in the central part of the heavy chains of the IgG and IgA immunoglobulin classes, which links these two chains by disulfide bonds. In particular, a hinge region forms a flexible linker between the Fab arms and the Fc part of a given antibody. It will also be understood that the length and flexibility of the hinge region may vary extensively among the IgG subclasses, and that a skilled person will be able to determine suitable hinges for use in the recombinant VLPs described herein.

A capsid fusion protein of the disclosure may comprise a linker (also referred to interchangeably herein as a linker peptide, a spacer or a spacer peptide). A linker may be used to join two or more functional domains of a fusion protein of the disclosure. For example, a linker may join the capsid protein to the TM protein domain. In another example, the TM protein

domain may be joined to the antigen by a linker, particularly where the TM protein domain and the antigen are from different viruses (e.g., from a S protein of a SARS-CoV-2 and a F or Pre F protein of a RSV). Use of linkers in fusion proteins is routine in the art, and any conventional linker protein may be used in fusion proteins of the disclosure, provided that the resulting fusion protein retains the desired functional properties of the antigen.

A linker may be a short peptide of up to about 30 amino acids, such as about 5-30 amino acids, about 5-25 amino acids, about 5-20 amino acids, about 10-20 amino acids, about 5-15 amino acids or about 10-15 amino acids in length. In some embodiments, the linker is about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19 or about 20 amino acids in length.

In an example, a rigid linker may be used in fusion proteins of the disclosure. Rigid linkers are conventionally used when it is necessary to keep a fixed distance between the different domains/portions of a fusion protein and to maintain their independent functions. Rigid linkers may also be used when the spatial separation of the fusion protein domains is critical to preserve the stability or bioactivity of the fusion proteins. An empirical rigid linker with the sequence of A(EAAAK)_nA ($n = 2-5$) displayed α -helical conformation, which is stabilized by Glu-Lys+salt bridges. A non-limiting example of a rigid linker is EAAAKEAAAKEAAAK (also referred to as (EAAAK)_h). Rigid linkers may be used for expression of fusion proteins of the disclosure in mammalian cells, such as HEK 293 cells.

In some embodiments, flexible linkers may be used in fusion proteins of the disclosure. Flexible linkers are conventionally used when the joined domains require a certain degree of movement or interaction. Flexible linkers usually comprise or consist of small amino acid residues, such as glycine, threonine, arginine, serine, asparagine, glutamine, alanine, aspartic acid, proline, glutamic acid, lysine, leucine and/or valine, particularly glycine, serine, alanine, leucine and/or valine. Flexible linkers comprising or consisting of glycine, serine and/or alanine are preferred, with glycine and serine being particularly preferred. Accordingly, the most commonly used flexible linkers have sequences consisting primarily of stretches of Gly and Ser residues ("GS" linker), which comprise a sequence of (Gly-Gly-Gly-Gly-Ser)_n. Non-limiting examples of GS linkers include GS₅; GS₁₀; GS₁₅; GS₂₀; and GS₂₅.

Antigens

Antigens suitable for use in the VLPs and compositions described herein will be apparent to the skilled person. In an example, the antigen is a spike (S) protein from a SARS-CoV-2, a F or Pre F protein from RSV or a HA or NA protein from influenza.

SARS-CoV-2 Antigens

In an example, the present disclosure provides for VLPs comprising a capsid fusion protein which includes an antigen from a severe acute respiratory syndrome coronavirus 2

(SARS-CoV-2), for example a spike (S) protein. In the context of the present disclosure, the antigen is therefore a pathogenic antigen. For example, the antigen of interest is an antigen protein, an immunogenic fragment and/or variant thereof which can induce an immune response in the subject.

The SARS-CoV-2 genome encodes for at least four main structural proteins: the spike (S), membrane (M), envelope (E), nucleocapsid (N) proteins and other accessory proteins which aid the replicative processes and facilitate entry into cells. The M protein is the most abundant component of the virus envelope, which directs the assembly of coronaviruses through interactions with all other structural proteins. The E protein is a small membrane protein or viroporin that is thought to promote budding of virus particles by pinching off cellular membrane surfaces. The S protein is a class I fusion protein that mediates attachment of SARS-CoV-2 to the major cell surface receptor human Angiotensin Converting Enzyme 2 (ACE2). Due to its exposed conformation on the surface of the virus, the S protein is highly immunogenic and is the main focus of current vaccine development. The N protein packages the RNA genome to form the nucleocapsid and whilst not necessarily required for envelope formation; it appears to play an important role in the assembly and stability of the complete virion, and in enhancement of VLP yields.

The S protein comprises three domains: (i) a large ectodomain; (ii) a transmembrane domain (which passes through the viral envelope in a single pass); and (iii) a short intracellular tail. The ectodomain consists of three receptor-binding subunits (3 x S1) and a trimeric stalk made of three membrane-fusion subunits (3 x S2). Thus, the SARS-CoV-2 S protein is a homotrimer. During virus entry, S1 binds to a receptor on the host cell surface for viral attachment, and S2 fuses the host and viral membranes, allowing viral genomes to enter host cells. Receptor binding and membrane fusion are the initial and critical steps in the coronavirus infection cycle. There is significant divergence in the receptors targeted by different CoVs.

The structure of the SARS-CoV-2 S protein is described, for example, in Cai et al. (Science (2020) 369:1586-1592)), which is herein incorporated by reference in its entirety. Each S1 subunit of a SARS-CoV-2 S protein comprises an N-terminal domain (NTD), receptor binding domain (RBD), two C terminal domains (CTDs). Prior to fusion with the host cell membrane, the S1 subunits of the SARS-CoV-2 S protein protect the S2 subunits. On binding to ACE2, the SARS-CoV-2 S protein refolds in a "jack-knife" manner, forming a long-central coiled coil and ultimately leading to membrane fusion and viral entry to a host cell.

Given the propensity of RNA viruses such as SARS-CoV-2 to mutate, the present inventors provide for VLPs that comprise S proteins that may include mutations found in different strains of SARS-CoV-2, such that the vaccine compositions find particular utility in the treatment of targeted strains of SARS-CoV-2 including the omicron strain of SARS-CoV-2.

The VLPs, compositions and vaccines of the present disclosure may also be useful for the treatment of variants of SARS-CoV-2 including B.1.1.7 SARS-CoV-2 strain (also known as

201/501Y.V1, which was first detected in the UK, now known as the Alpha variant); the B.1.351 SARS-CoV-2 strain (also known as 20H/501.V2, which was first detected in South Africa, now known as the Beta variant), the PI SARS-CoV-2 strain (also known as 20J/501 Y.V3, which was first detected in Japan and Brazil, now known as the Gamma variant), the B1.427 and B1.429 SARS-CoV-2 strains (first detected in California, now known as the Epsilon variant), and/or the B.1.617.2 SARS-CoV-2 strain (which was first detected in India, now known as the Delta variant). The VLPs, compositions and vaccines of the present disclosure may also be useful for the treatment of the Wuhan (original) strain of SARS-CoV-2.

According to the CDC (SARS-CoV-2 Variant Classifications and Definitions ([cdc.gov](https://www.cdc.gov/))), the Alpha variant has been found to comprise the following mutations to the S protein: 69deletion, 70deletion, 144deletion, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, and (K1191N*) with the key mutations being deletion of residues 69/70 and 144Y, as well as N501Y, A570D, D614G and P681H substitutions. The Beta variant has been found to comprise the following mutations: D80A, D215G, 241deletion, 242deletion, 243deletion, K417N, E484K, N501Y, D614G, and A701V with the key mutations being K417N, E484K, N501Y and D614G substitutions. The Gamma variant has been found to comprise the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I with the key mutations being E484K, K417N/T, N501Y and D614G. The Delta variant has been found to comprise the following mutations: T19R, (G142D*), 156deletion, 157deletion, R158G, L452R, T478K, D614G, P681R, and D950N with the key mutations being L452R, E484Q and T478K. The Epsilon variant has been found to comprise the following mutations: S131, W152C, 30 L452R, D614G with the key mutation being L452R. Thus, the present disclosure encompasses VLPs comprising antigens from a S protein which include or more or all of the above mutations.

In one example, the VLP comprises an antigen from each of the S protein, M protein, and E protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and M protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and E protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the E protein and M protein of the omicron strain of a SARS-CoV-2. In an example, the omicron variant may be BA.1 or BA.2.

Influenza

Influenza, also known as "the flu", is an infectious disease caused by an influenza virus. It will be apparent to the skilled person that there are currently four influenza viruses – A, B, C and D. Influenza A virus is the most common flu virus infecting humans, animals, and birds, whilst influenza B virus infection mostly occurs in humans. Infection of influenza C virus does

not cause any severe symptom in human or mammals and influenza D, to date, has only infected pigs and cattle.

In one example of the VLPs of the present disclosure, the antigen is from an influenza A virus strain. For example, the antigen is an influenza A virus hemagglutinin (HA) protein, a neuraminidase (NA) protein, a matrix (M) protein, a nucleoprotein (NP), a non-structural (NS) protein, or an immunogenic fragment or variant thereof. In one example, the antigen is an influenza A hemagglutinin (HA) subtype H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 and/or an influenza A neuraminidase (NA) subtype N1, N2, N3, N4, N5, N6, N7, N8 or N9 and/or an influenza A matrix (M) protein subtype M1 or M2 and/or an influenza A non-structural (NS) protein subtype NS1 or NS2.

The skilled person will be aware that pandemic strains of the influenza virus are commonly H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strains. For example, H1N1, H2N2, H3N2, H5N1, H5N3, H6N1, H7N2, H7N3, H7N7, H7N9 and H9N2, strains. In one example, the antigen is a H1N1 antigen from a A/Delaware/55/2019 virus strain. In one example, the antigen is a H1 antigen from a A/Delaware/55/2019 virus strain.

In one example, the antigen is a H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strain. For example, the antigen is a H1 hemagglutinin, or a H2 hemagglutinin, or a H3 hemagglutinin, or a H5 hemagglutinin, or a H6 hemagglutinin, or a H7 hemagglutinin or a H9 hemagglutinin. For example, the antigen is a H5 subtype influenza A virus strain (i.e., a H5 hemagglutinin). In one example, the H5 hemagglutinin is an A/turkey/Turkey/1/2005 virus strain. In one example, the H3 hemagglutinin is an A/Delaware/39/2019 virus strain.

In one example, the antigen is an N1, N2, N3, N7 or N9 subtype influenza A virus strain. For example, the antigen is a N1 neuraminidase, or a N2 neuraminidase, or a N3 neuraminidase, or a N7 neuraminidase, or a N9 neuraminidase. For example, the antigen is a N1 neuraminidase subtype influenza A virus strain. In one example, the N1 neuraminidase is an A/turkey/Turkey/1/2005 strain. In one example, the N2 neuraminidase is an A/Delaware/39/2019 virus strain.

In one example, the antigen is an influenza B virus strain. The skilled person will be aware that influenza B viruses are not divided into subtypes but are classified into two lineages, namely, B/Yamagata and B/Victoria.

In one example, the antigen is a B/Yamagata influenza B virus strain. For example, the influenza B virus strain is a B/Singapore/INFTT 16 0610/16 (By) virus strain. In another example, the antigen is from a B/Victoria influenza B virus strain.

Respiratory syncytial virus (RSV)

RSV is an enveloped non-segmented negative-strand RNA virus in the family Paramyxoviridae, genus *Pneumovirus*. To infect a host cell, paramyxoviruses such as RSV, like other enveloped viruses such as influenza virus require fusion of the viral membrane with a host

cell's membrane. For RSV, the conserved fusion protein (RSV-F glycoprotein) fuses the viral and cellular membranes by coupling irreversible protein refolding with juxtaposition of the membranes. Based on paramyxovirus studies, the RSV-F protein initially folds into a metastable pre-fusion conformation. During cell entry, the pre-fusion conformation undergoes refolding and conformational changes to its stable post-fusion conformation.

In an example, the antigen is from a RSV. For example, the antigen is a RSV surface glycoprotein selected from the Fusion (F), Glycoprotein (G), Small Hydrophobic protein (SH), the matrix proteins M and M2, the nucleocapsid proteins N, P and L, and the nonstructural proteins NS1 and NS2. In certain examples, the antigen is an RSV-F antigen. In another example, the antigen is from a prefusion (Pre F) protein of a RSV. The F-protein presents two different conformations, a lollipop-shaped Pre F, present on the virus surface before virus–cell interaction, and a crutch-shaped postfusion (Post F) state which is acquired following the fusion between the virus and cell membrane or by unknown mechanisms that spontaneously initiate the rearrangement from the highly metastable preF into the energetically favorable postF conformation. The two forms are antigenically distinct, and both are considered as potential vaccine candidates.

The F glycoprotein of RSV is a type I single-pass integral membrane protein having four general domains: N-terminal ER-translocating signal sequence (SS), ectodomain (ED), transmembrane domain (TM), and a cytoplasmic tail (CT). CT contains a single palmitoylated cysteine residue. The sequence of F protein is highly conserved among RSV isolates but evolves over time. Unlike most paramyxoviruses, the F protein in RSV can mediate entry and syncytium formation independent of the other viral proteins (HN is usually necessary in addition to F in other paramyxoviruses).

The RSV-F glycoprotein is translated from mRNA into an approximately 574 amino acid protein designated F0. Post-translational processing of F0 includes removal of an N-terminal signal peptide by a signal peptidase in the endoplasmic reticulum. F0 is also cleaved at two sites (approximately 109/110 and approximately 136/137) by cellular proteases (in particular furin) in the trans-Golgi. This cleavage results in the removal of a short intervening sequence and generates two subunits designated F1 (~50 kDa; C-terminal; approximately residues 137-574) and F2 (~20 kDa; N-terminal; approximately residues 1-109) that remain associated with each other. F1 contains a hydrophobic fusion peptide at its N-terminus and also two amphipathic heptad-repeat regions (HRA and HRB). HRA is near the fusion peptide and HRB is near the transmembrane domain. Three F1-F2 heterodimers are assembled as homotrimers of F1-F2 in the virion.

RSV-F antigens suitable for inclusion in the VLPs or compositions described herein include RSV-F glycoprotein and RSV-F glycoprotein variants. Suitable RSV-F glycoprotein variants include, for example, full length F protein and truncated variants such as soluble ectodomains, each optionally containing one or more mutations, such as furin-cleavage mutations,

trypsin-cleavage mutations, fusion peptide mutations (e.g., deletions in whole or in part), mutations that stabilize the HRB trimer, and mutations that destabilize the HRA trimer.

Full length and truncated RSV-F glycoproteins, including those with one or more such mutations in a variety of combinations are well known in the art and are disclosed for example in WO2011/008974, the disclosure of which is incorporated herein by reference in its entirety.

In an example, the composition described herein comprises additional antigens. In an example, the additional antigen is a virus, bacteria, a fungus or a protozoan.

Viral antigens

In one example, the VLPs of the present disclosure further comprise an additional capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:

- (a) a capsid protein from a non-enveloped virus;
- (b) a transmembrane (TM) protein domain; and
- (c) an antigen protein,

wherein the capsid protein is encapsulated within the lipid bilayer.

In an example, the additional capsid fusion protein is different to the first capsid fusion protein described herein and comprises a different TM protein domain and/or antigen protein. In an example the additional antigen protein is a viral antigen protein.

Additional viral antigens will be apparent to the skilled person and include, for example, proteins and peptides from a *Orthomyxoviruses* (e.g., Influenza A, B and C), *Paramyxoviridae* viruses (Pneumoviruses (e.g., Respiratory syncytial virus (RSV), Bovine respiratory syncytial virus, Pneumonia virus of mice, and Turkey rhinotracheitis virus), Paramyxovirus types 1-4 (PIV), Mumps, Sendai viruses, Simian virus 5)), Bovine parainfluenza virus, Nipahvirus, Henipavirus and Newcastle disease virus), Poxviridae (e.g., Variola vera, including but not limited to, Variola major and Variola minor, Metapneumoviruses, such as human metapneumovirus (hMPV) and avian metapneumoviruses (aMPV)), Morbilliviruses (e.g., Measles), Picornaviruses (e.g., Enteroviruses, Rhinoviruses, Heparnavirus, Parechovirus, Cardioviruses and Aphthoviruses), Enteroviruses (e.g., Poliovirus types 1, 2 or 3, Coxsackie A virus types 1 to 22 and 24, Coxsackie B virus types 1 to 6, Echovirus (ECHO) virus types 1 to 9, 11 to 27 and 29 to 34 and Enterovirus 68 to 71), Bunyaviruses (e.g., California encephalitis virus), Phlebovirus (e.g., Rift Valley Fever virus), Nairovirus (e.g., Crimean-Congo hemorrhagic fever virus), Heparnaviruses (e.g., Hepatitis A virus (HAV)), Togaviruses (e.g., Rubivirus, an Alphavirus, or an Arterivirus), Flaviviruses (e.g., Tick-borne encephalitis (TBE) virus, Dengue (types 1, 2, 3 or 4) virus, Yellow Fever virus, Japanese encephalitis virus, Kyasanur Forest Virus, West Nile encephalitis virus, St. Louis encephalitis virus, Russian spring-summer encephalitis virus, Powassan encephalitis virus), Pestiviruses (e.g., Bovine viral diarrhea (BVDV), Classical swine fever (CSFV) or Border disease (BDV)), Hepadnaviruses (e.g., Hepatitis B virus, Hepatitis C virus), Rhabdoviruses (e.g., Lyssavirus (Rabies virus) and Vesiculovirus (VSV)), Caliciviridae

(e.g., Norwalk virus, and Norwalk-like Viruses (e.g., Hawaii Virus and Snow Mountain Virus); Coronaviruses (e.g., severe acute respiratory syndrome (SARS) coronavirus (SARS-CoV), SARS coronavirus 2 (SARS-CoV-2), Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV), Avian infectious bronchitis (IBV), Mouse hepatitis virus (MHV), and Porcine transmissible gastroenteritis virus (TGEV)), Retroviruses (e.g., Oncovirus, a Lentivirus or a Spumavirus), Reoviruses (e.g., Orthoreo virus, a Rotavirus, an Orbivirus, or a Coltivirus), Parvoviruses (e.g., Parvovirus B 19), Delta hepatitis virus (HDV), Hepatitis E virus (HEV), Human Herpesviruses (e.g., Herpes Simplex Viruses (HSV), Varicella-zoster virus (VZV), Epstein-Barr virus (EBV), Cytomegalovirus (CMV), Human Herpesvirus 6 (HHV6), Human Herpesvirus 7 (HHV7), and Human Herpesvirus 8 (HHV8)), Papovaviruses (e.g., Papillomaviruses and Polyomaviruses), Adenoviruses and Arenaviruses.

In one example, the additional viral antigen is from a parainfluenza virus.

In one example, the additional viral antigen is from a metapneumovirus.

In one example, the additional viral antigen is from a rhinovirus.

In one example, the additional viral antigen is from a coronavirus.

In one example, the additional viral antigen is from an adenovirus.

In one example, the additional viral antigen is from a bocavirus.

In one example, the additional antigen is from a single strain of an influenza virus (i.e., monovalent) or from multiple strains (i.e., multivalent).

In one example, the additional antigen is an influenza A, B and/or C virus strain.

In one example, the additional antigen is an influenza A virus strain. For example, the antigen is an influenza A virus hemagglutinin (HA) protein, a neuraminidase (NA) protein, a matrix (M) protein, a nucleoprotein (NP), a non-structural (NS) protein, or an immunogenic fragment or variant thereof. In one example, the additional antigen is an influenza A hemagglutinin (HA) subtype H1, H2, H3, H4, H5, H6, H7, H8, H9, H10, H11, H12, H13, H14, H15 or H16 and/or an influenza A neuraminidase (NA) subtype N1, N2, N3, N4, N5, N6, N7, N8 or N9 and/or an influenza A matrix (M) protein subtype M1 or M2 and/or an influenza A non-structural (NS) protein subtype NS1 or NS2.

The skilled person will be aware that pandemic strains of the influenza virus are commonly H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strains. For example, H1N1, H2N2, H3N2, H5N1, H5N3, H6N1, H7N2, H7N3, H7N7, H7N9 and H9N2, strains.

In one example, the additional antigen is from a H1, H2, H3, H5, H6, H7 or H9 subtype influenza A virus strain. For example, the additional antigen is a H1 hemagglutinin, or a H2 hemagglutinin, or a H3 hemagglutinin, or a H5 hemagglutinin, or a H6 hemagglutinin, or a H7 hemagglutinin or a H9 hemagglutinin. For example, the additional antigen is from a H5 subtype influenza A virus strain (i.e., a H5 hemagglutinin). In one example, the H5 hemagglutinin is an A/turkey/Turkey/1/2005 virus strain. In one example, the H3 hemagglutinin is an A/Delaware/39/2019 virus strain.

In one example, the additional antigen is N1, N2, N3, N7 or N9 subtype influenza A virus strain. For example, the additional antigen is a N1 neuraminidase, or a N2 neuraminidase, or a N3 neuraminidase, or a N7 neuraminidase, or a N9 neuraminidase. For example, the additional antigen is a N1 neuraminidase subtype influenza A virus strain. In one example, the N1 neuraminidase is an A/turkey/Turkey/1/2005 strain. In one example, the N2 neuraminidase is an A/Delaware/39/2019 virus strain.

Infections such as influenza and coronavirus infection are leading causes of ARDS. Accordingly, in one example of the present disclosure, the ARDS is associated with an influenza, RSV or a SARS-CoV-2 infection. In one example, the ARDS is associated with a SARS-CoV-2 infection. Thus, a skilled person will understand that antigens targeting a SARS-CoV-2 infection or influenza, including those listed above, may be antigens suitable for the treatment of ARDS.

Bacterial antigens

In one example, the additional antigen of the present disclosure is a bacterial antigen.

Bacterial antigens will be apparent to the skilled person and include, for example, proteins and peptides from a *Neisseria meningitides*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, *Bordetella pertussis*, *Burkholderia sp.* (e.g., *Burkholderia mallei*, *Burkholderia pseudomallei* and *Burkholderia cepacia*), *Staphylococcus aureus*, *Haemophilus influenzae*, *Clostridium tetani* (Tetanus), *Clostridium perfringens*, *Clostridium botulinum*, *Corynebacterium diphtheriae* (Diphtheria), *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Coxiella burnetii*, *Brucella sp.* (e.g., *B. abortus*, *B. canis*, *B. melitensis*, *B. neotomae*, *B. ovis*, *B. suis* and *B. pinnipediae*), *Francisella sp.* (e.g., *F. novicida*, *F. philomiragia* and *F. tularensis*), *Streptococcus agalactiae*, *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum* (Syphilis), *Haemophilus ducreyi*, *Enterococcus faecalis*, *Enterococcus faecium*, *Helicobacter pylori*, *Staphylococcus saprophyticus*, *Yersinia enterocolitica*, *E. coli*, *Bacillus anthracis* (anthrax), *Yersinia pestis* (plague), *Mycobacterium tuberculosis*, *Rickettsia*, *Listeria*, *Chlamydia pneumoniae*, *Vibrio cholerae*, *Salmonella typhi* (typhoid fever), *Borrelia burgdorferi*, *Porphyromonas sp.*, *Klebsiella sp.*

Fungal antigens

In one example, the additional antigen of the present disclosure is a fungal antigen.

Fungal antigens that can be encoded by a RNA according to the present disclosure or provided in the form of a polypeptide will be apparent to the skilled person and include, for example, proteins and peptides from *Dermatophytes* (including *Epidermophyton floccosum*, *Microsporum audouinii*, *Microsporum canis*, *Microsporum distortum*, *Microsporum equinum*, *Microsporum gypsum*, *Microsporum nanum*, *Trichophyton concentricum*, *Trichophyton equinum*, *Trichophyton gallinae*, *Trichophyton gypseum*, *Trichophyton megnini*, *Trichophyton mentagrophytes*, *Trichophyton quinckeum*, *Trichophyton rubrum*, *Trichophyton schoenleini*,

Trichophyton tonsurans, *Trichophyton verrucosum*, *T. verrucosum* var. *album*, var. *discoideus*, var. *ochraceum*, *Trichophyton violaceum*, and/or *Trichophyton faviforme*), *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus sydowi*, *Aspergillus flavatus*, *Aspergillus glaucus*, *Blastoschizomyces capitatus*, *Candida albicans*, *Candida enolase*, *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis*, *Candida stellatoidea*, *Candida kusei*, *Candida parakwsei*, *Candida lusitanae*, *Candida pseudotropicalis*, *Candida guilliermondi*, *Cladosporium carrionii*, *Coccidioides immitis*, *Blastomyces dermatidis*, *Cryptococcus neoformans*, *Geotrichum clavatum*, *Histoplasma capsulatum*, *Klebsiella pneumoniae*, *Microsporidia*, *Encephalitozoon* spp., *Septata intestinalis* and *Enterocytozoon bieneusi*.

Protazoan antigens

In one example, the additional antigen of the present disclosure is a protazoan antigen.

Protazoan antigens will be apparent to the skilled person and include, for example, proteins and peptides from *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, *Cyclospora cayatanensis* and *Toxoplasma*.

Polynucleotides

The present disclosure also provides polynucleotides that when expressed under sufficient conditions, encode a VLP disclosed herein and are suitable for forming a composition or vaccine for treating an infection such as a viral infection. The term polynucleotide encompasses both DNA and RNA sequences. Herein, the terms "nucleic acid", "nucleic acid molecule" and "polynucleotide" are used interchangeably. Thus, the antigens derived from SARS-CoV-2, influenza or RSV, as well as the capsid fusion protein may be encoded or expressed by DNA or RNA comprised within one or more expression cassettes or vectors.

By way of non-limiting example, where the VLP comprises different capsid fusion proteins which comprise different protein antigens (e.g., from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and from RSV), said more than one antigen may be expressed by a monocistronic polynucleotide, or each of said antigens may be expressed by polycistronic polynucleotides.

The one or more polynucleotides (e.g. a DNA or RNA) encoding the capsid fusion protein may be optimised for expression in a cell. The term "optimised" as used herein relates to optimisation for expression of the capsid fusion protein, and includes both codon optimisation and/or other modifications to the polynucleotide (both in terms of the nucleic acid sequence and other modifications) which increase the level and/or duration of expression of the capsid fusion protein from the polynucleotide within the cell.

The one or more polynucleotides (e.g. a DNA or RNA) according to the disclosure may be comprised in an expression vector to facilitate expression of the capsid fusion protein.

Typically, in such an expression construct said one or more polynucleotide is operably linked to a suitable promoter(s). The one or more polynucleotide may be linked to a suitable terminator sequence(s). The one or more polynucleotide may also be linked to both a promoter(s) and terminator(s). Suitable promoter and terminator sequences are well known in the art.

The one or more polynucleotide (e.g. DNA or RNA) encoding the capsid fusion protein may additionally comprise a leader sequence(s). Any suitable leader sequence may be used, including conventional leader sequences known in the art. Suitable leader sequences include human tissue plasminogen activator leader sequence (tPA), which is routinely used in viral and DNA based vaccines and for protein vaccines to aid secretion from mammalian cells.

One or more viral vectors, expression vectors or DNA vectors (or DNA plasmids) may comprise one or more polynucleotides encoding a capsid fusion protein described herein. Preferably, said one or more viral vector or DNA vector (or DNA plasmid) encodes at least one antigen as described herein. Multiple capsid fusion proteins may be expressed by a single viral vector or DNA vector (or DNA plasmid) by multiple viral vectors or DNA vectors (or DNA plasmids) or a combination thereof. By way of non-limiting example, where different capsid fusion proteins are contemplated, said capsid fusion proteins may be expressed by a single viral vector or DNA vector (or DNA plasmid) or each capsid fusion protein may be expressed by a separate viral vector or DNA vector (or DNA plasmid).

The one or more vector(s) may be a DNA vector, such as a DNA plasmid. The one or more vector(s) may be an RNA vector, such as a mRNA vector or a self-amplifying RNA vector. The one or more DNA and/or RNA vector(s) of the disclosure is typically capable of expression in eukaryotic cells, particularly any host cell type described herein.

Typically the DNA and/or RNA vector(s) are capable of expression in a human, *e. coli* or yeast cell. The one or more vector may be a phage vector, such as an AAV/phage hybrid vector as described in Hajitou et al., Cell 2006; 125(2) pp. 385-398; herein incorporated by reference.

The nucleic acid molecules and vectors of the disclosure may be made using any suitable process known in the art. Thus, the nucleic acid molecules may be made using chemical synthesis techniques. Alternatively, the nucleic acid molecules and vectors of the disclosure may be made using molecular biology techniques.

Methods of Production

Suitable methods for the production of a VLP of the present disclosure will be apparent to the skilled person and/or described herein.

Typically, the plasmid DNA is produced by inserting the polynucleotide sequence encoding at least one antigen into a DNA vector. Suitable DNA vectors for use will be apparent to the skilled person, and the polynucleotide sequences of the present disclosure can be purchased from any commercial supplier. Insertion of the nucleotide sequence(s) into the DNA vector may be performed using standard methods in the art.

In one example, a protein antigen described herein is produced using a plasmid DNA. The skilled person will understand that plasmid DNA is relatively stable. Briefly, competent bacterial cells (e.g., *Escherichia coli*) cells are transformed with a DNA plasmid encoding the a protein antigen described herein. Individual bacterial colonies are isolated and the resultant plasmid DNA amplified in *E. coli* cultures.

In one example, the plasmid DNA is isolated following fermentation. For example, the plasmid DNA is isolated using a commercially available kit (e.g., Maxiprep DNA kit), or other routine methods known to the skilled person. Following isolation, plasmid DNA is linearized by restriction digest (i.e., using a restricting enzyme). Restriction enzymes are removed using methods known in the art, including for example phenol/chloroform extraction and ethanol precipitation.

Compositions

The present disclosure provides an immunogenic composition comprising a VLP of the present disclosure. In an example, the immunogenic composition is a vaccine. The present disclosure also provides a pharmaceutical composition comprising an immunogenic composition of the present disclosure and a pharmaceutically acceptable carrier.

In an example, the VLPs described herein may be administered in a composition comprising an adjuvant for enhancing immunogenicity. In an example, the adjuvant is selected from the group consisting of Freund's adjuvant, incomplete Freund's adjuvants, aluminum phosphate, aluminum hydroxide, GMCSF, BCG, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, monophosphoryl lipid A (MPL), RIBI, MPL, trehalose dimycolate (TDM), Novasomes®, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide, MHC antigens, PolyI:C, MF59, glycolipid analogs, octadecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, ODA, cytokines, and other adjuvants and derivatives thereof. In an example, the adjuvant is MF59. In an example, MF59 is administered at the same time as the administration of a VLP, composition or vaccine of the disclosure. In another example, MF59 is administered sequentially to, preceding, or proceeding the administration of a VLP, composition or vaccine of the disclosure.

It will be apparent to the skilled person and/or described herein, that the VLP of the present disclosure may be present as a VLP or in combination with lipids, polymers or other delivery system that facilitates entry into the cells.

Delivery systems

In one example, the pharmaceutical composition of the present disclosure further comprises a lipid nanoparticle (LNP) and/or a polymeric microparticle. For example, the VLP is encapsulated in, bound to or adsorbed on a LNP and/or a polymeric microparticle.

Lipid Nanoparticles

In one example, the pharmaceutical composition of the present disclosure further comprises a LNP.

It will be apparent that the term “lipid nanoparticle” or “LNP” shall be understood to refer to any lipid composition, including, but not limited to, liposomes or vesicles, where an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar), micelle-like lipid nanoparticles having a non-aqueous core and solid lipid nanoparticles. Methods of preparing a LNP are known to the skilled person and/or described herein. In one example, LNP are prepared using a staggered herribone mixer. For example, as described in US patent application 20120276209. In another example, liposomes are prepared using a microfluidic device. For example, as described in WO2018220553.

Lipid nanoparticles suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein. For example, the LNP comprises an ionisable lipid.

As used herein, the term “ionisable lipid” or “ionisable lipids” shall refer to a lipid having at least one protonatable or deprotonatable group. For example, the lipid is positively charged at a pH at or below physiological pH (e.g. pH 7.4), and neutral at a second pH (e.g. at or above physiological pH). For example, the lipid is a cationic lipid.

Suitable ionisable lipids can have an anionic, cationic or zwitterionic hydrophilic head group. Exemplary phospholipids (anionic or zwitterionic) for use in the present disclosure include, for example, phosphatidylethanolamines, phosphatidylcholines, phosphatidylserines, and phosphatidylglycerols. In one example, the lipid is a cationic lipid. Exemplary cationic lipids include, but are not limited to, dioleoyl trimethylammonium propane (DOTAP), 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 1,2-dioleoyloxy-N,N-dimethyl-3-aminopropane (DODMA), 1,2-dilinoleoyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 2,5-bis((9z,12z)-octadeca-9,12,dien-1-yloxy)benzyl-4-(dimethylamino)butanoate (LKY750). In one example, the phospholipid is 2,5-bis((9z,12z)-octadeca-9,12,dien-1-yloxy)benzyl-4-(dimethylamino)butanoate (LKY750). Exemplary zwitterionic lipids include, but are not limited to, acyl zwitterionic lipids and ether zwitterionic lipids, such as dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC) and dodecylphosphocholine. The lipids can be saturated or unsaturated.

Lipid moieties suitable for use in the LNP will be apparent to the skilled person and include, for example, a fatty acid, an isoprenoid and combinations thereof. In one example, the lipid moiety is selected from the group consisting of an isoprenoid, a triglyceride, a phospholipid, a cholesteryl ester and combinations thereof.

In one example, the lipid nanoparticle additionally comprises a PEG-lipid, a sterol structural lipid and/or a neutral lipid. In one example, the lipid nanoparticle does not comprise a cationic lipid.

PEG-lipids

In one example, the present disclosure provides a LNP comprising a PEGylated lipid.

It will be apparent to the skilled person that reference to a PEGylated lipid is a lipid that has been modified with polyethylene glycol. Exemplary PEGylated lipids include, but are not limited to, PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols. For example, a PEG lipid includes PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, a PEG-DSPE lipid and combinations thereof.

Neutral lipids

In one example, the present disclosure provides a LNP comprising a neutral lipid.

Suitable neutral or zwitterionic lipids for use in the present disclosure will be apparent to the skilled person and include, for example, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosahexaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin. The lipids can be saturated or unsaturated.

Structural lipids

In one example, the present disclosure provides a LNP comprising a structural lipid.

Exemplary structural lipids include, but are not limited to, cholesterol fecosterol, sitosterol, campesterol, stigmasterol, brassicasterol, ergosterol, tomatidine, tomatine, ursolic acid and alpha-tocopherol.

In one example, the structural lipid is a sterol. For example, the structural lipid is cholesterol. In another example, the structural lipid is campesterol.

Polymeric microparticles

In one example, the pharmaceutical composition of the present disclosure further comprises a polymeric microparticle.

The skilled person will be aware that various polymers can form microparticles to encapsulate or adsorb the protein antigens or VLPs of the present disclosure. It will be apparent that use of a substantially non-toxic polymer means that particles are safe, and the use of a biodegradable polymer means that the particles can be metabolised after delivery to avoid long-term persistence. Useful polymers are also sterilisable, to assist in the preparation of pharmaceutical grade formulations.

Exemplary non-toxic and biodegradable polymers include, but are not limited to, poly(α -hydroxy acids), polyhydroxy butyric acids, polylactones (including polycaprolactones), polydioxanones, polyvalerolactone, polyorthoesters, polyanhydrides, polycyanoacrylates, tyrosine-derived polycarbonates, polyvinyl- pyrrolidinones or polyester-amides, and combinations thereof.

Pharmaceutically acceptable carrier

Suitably, in compositions or methods for administration of the VLP, vaccine or composition of the disclosure to a subject, the VLP, vaccine or composition is combined with a pharmaceutically acceptable carrier as is understood in the art. Accordingly, one example of the present disclosure provides a composition (e.g., a pharmaceutical composition) comprising the VLP of the disclosure (and any delivery system e.g. LNP) combined with a pharmaceutically acceptable carrier.

In general terms, by “carrier” is meant a solid or liquid filler, binder, diluent, encapsulating substance, emulsifier, wetting agent, solvent, suspending agent, coating or lubricant that may be safely administered to any subject, e.g., a human. Depending upon the particular route of administration, a variety of acceptable carriers, known in the art may be used, as for example described in Remington's Pharmaceutical Sciences (Mack Publishing Co. N.J. USA, 1991).

A VLP, composition or vaccine of the present disclosure is useful for parenteral, topical, oral, or local administration, intramuscular administration, aerosol administration, or transdermal administration, for prophylactic or for therapeutic treatment. In one example, the VLP, composition or vaccine is administered parenterally, such as intramuscularly, subcutaneously or intravenously. For example, the RNA is administered intramuscularly.

Formulation of a VLP, composition or vaccine of the present disclosure to be administered will vary according to the route of administration and formulation (e.g., solution, emulsion, capsule) selected. An appropriate pharmaceutical composition comprising a VLP, composition or vaccine to be administered can be prepared in a physiologically acceptable carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated

Ringer's or fixed oils. A variety of appropriate aqueous carriers are known to the skilled artisan, including water, buffered water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol), dextrose solution and glycine. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. 1980). The compositions can optionally contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents and toxicity adjusting agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate. The VLP, composition or vaccine can be stored in the liquid stage or can be lyophilized for storage and reconstituted in a suitable carrier prior to use according to art-known lyophilization and reconstitution techniques.

The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures known to the skilled artisan, and will depend on the ultimate pharmaceutical formulation desired.

Upon formulation, compositions of the present disclosure will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically/prophylactically effective. The dosage ranges for the administration of the molecule of the disclosure are those large enough to produce the desired effect. For example, the composition comprises an effective amount of the VLP, composition or vaccine of the present disclosure. In one example, the composition comprises a therapeutically effective amount of the VLP, composition or vaccine of the present disclosure. In another example, the composition comprises a prophylactically effective amount of the VLP, composition or vaccine of the present disclosure.

The dosage should not be so large as to cause adverse side effects. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any complication.

Dosage can vary from about 0.1 mg/kg to about 300 mg/kg, e.g., from about 0.2 mg/kg to about 200 mg/kg, such as, from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days.

In some examples, the VLP, composition or vaccine of the present disclosure is administered at an initial (or loading) dose which is higher than subsequent (maintenance doses). For example, the VLP, composition or vaccine of the present disclosure is administered at an initial dose of between about 10mg/kg to about 30mg/kg. The VLP is then administered at a maintenance dose of between about 0.0001mg/kg to about 10mg/kg. The maintenance doses may be administered every 7-35 days, such as, every 7 or 14 or 28 days.

In some examples, a dose escalation regime is used, in which the VLP, composition or vaccine of the present disclosure is initially administered at a lower dose than used in subsequent doses. This dosage regime is useful in the case of subject's initially suffering adverse events.

In the case of a subject that is not adequately responding to treatment, multiple doses in a week may be administered. Alternatively, or in addition, increasing doses may be administered.

A subject may be retreated with the VLP, composition or vaccine of the present disclosure, by being given more than one exposure or set of doses, such as at least about two exposures of the mRNA, for example, from about 2 to 60 exposures, and more particularly about 2 to 40 exposures, most particularly, about 2 to 20 exposures.

In one example, any retreatment may be given when signs or symptoms of disease return.

In one example, any retreatment may be given when there are no signs or symptoms of disease return.

In another example, any retreatment may be given at defined intervals. For example, subsequent exposures may be administered at various intervals, such as, for example, about 3-4 weeks, or 4-12 weeks, or 24-28 weeks, or 48-56 weeks or longer. For example, such exposures are administered at intervals each of about 3-4 weeks, or 4-8 week, or 4-12 weeks, or 24-26 weeks or about 38-42 weeks, or about 50-54 weeks.

In another example, for subjects experiencing an adverse reaction, the initial (or loading) dose may be split over numerous days in one week or over numerous consecutive days.

Administration of the VLP, composition or vaccine of the present disclosure according to the methods of the present disclosure can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the VLP may be essentially continuous over a preselected period of time or may be in a series of spaced doses, e.g., either during or after development of a condition.

Screening Assays

Virus-like particle (VLP) antigens

In one example, the composition comprising the VLP is assessed for expression of the antigens. For example, antigen expression is detected using antibodies against the S protein of a SARS-CoV-2, a Pre F or F protein of a RSV or a HA or NA protein of an influenza virus. In one example, the number of cells positive for antigen expression is measured by e.g., fluorescence-activated cell sorting (FACS). In another example the mean fluorescence intensity (MFI) is determined using e.g., FACS.

Quantification of Virus-like particle (VLP) release

In one example, the composition comprising VLPs is assessed for formation and release of VLPs from cells. For example, VLPs release from cells is analysed using antibodies against

VLP antigens. In a further example, association between VLP antigens is determined using antibody-mediated co-immunoprecipitation and/or detection of the VLP antigens in the co-immunoprecipitation sample by, for example, Western blot analysis.

Microneutralization Assay

In one example, the composition comprising the VLP is assessed for antibody responses. For example, the composition comprising the VLP is assessed using a microneutralisation assay. Methods of performing a microneutralization assay will be apparent to the skilled person. In one example, the microneutralization assay is a short form assay. For one example, a virus fluorescent focus-based microneutralization assay is performed. In another example, the microneutralization assay is a long form assay.

Hemagglutination inhibition (HAI) assay

In one example, the VLP is assessed for antibody responses. For example, the VLP is assessed using a hemagglutination inhibition (HAI) assay. Methods of performing a HAI assay will be apparent to the skilled person and/or described, for example, in WHO (2011) *Manual for the laboratory diagnosis and virological surveillance of influenza*: WHO Press, World Health Organization.

Antigen Specific T cell Responses

In one example, the composition comprising the VLP (naked and/or formulated) is assessed for its ability to induce antigen specific T cell responses. Methods of assessing induction of antigen specific T cell responses will be apparent to the skilled person and/or are described herein.

For example, antigen-specific T cell detection is performed on splenic cultures. Briefly, splenocyte cultures are established in T cell medium and cell cultures are either stimulated with antigenic peptides or unstimulated. In one example, antigen-specific T cell responses are determined using flow cytometry.

Methods of Treatment or Prevention

The present disclosure provides methods of using the immunogenic composition or the pharmaceutical composition of the present disclosure as a vaccine.

The present disclosure also provides methods of treating or preventing a disease or condition in a subject comprising administering the immunogenic composition or the pharmaceutical composition of the present disclosure. For example, the disease or condition is a respiratory virus infection, such as influenza, a SARS-CoV-2 infection, COVID-19, or respiratory syncytial virus (RSV). In another example, the disease or condition is acute respiratory distress syndrome (ARDS).

Influenza

Influenza, also known as "the flu", is an infectious disease caused by an influenza virus. Symptoms can be mild to severe and the most common symptoms include high fever, runny nose, sore throat, muscle and joint pain, headache, coughing, and feeling tired. Symptoms typically begin two days after exposure to the virus and most last less than a week. Complications of influenza may include viral pneumonia, secondary bacterial pneumonia, sinus infections, and worsening of previous health problems such as asthma or heart failure. Viral pneumonia may also lead to acute respiratory distress syndrome (ARDS).

It will be apparent to the skilled person that there are currently four influenza viruses – A, B, C and D. Influenza A virus is the most common flu virus infecting humans, animals, and birds, whilst influenza B virus infection mostly occurs in humans. Infection of influenza C virus does not cause any severe symptom in human or mammals and influenza D, to date, has only infected pigs and cattle.

Thus, in some examples of the present disclosure, the subject has an influenza virus infection. In one example, the subject has influenza. In particular, the influenza is associated with ARDS. In one example, the methods of the present disclosure can be used to treat or prevent ARDS in a subject suffering from an influenza virus infection. In one example, the methods of the present disclosure can be used to treat or prevent ARDS in a subject suffering from influenza.

In an example, the methods described herein comprise the identification of a subject having or suspected of having influenza. In this example, the subject may have one or more of the above symptoms and may be classified as having mild or severe influenza.

Coronavirus Disease 2019 (COVID-19)

The present disclosure provides, for example, methods of treating or preventing COVID-19. The present disclosure also provides, for example, methods of treating or preventing SARS-CoV-2 infection. In some examples of the present disclosure the subject has a SARS-CoV-2 infection but does not have clinically diagnosed COVID-19.

COVID-19 is an infectious disease caused by SARS-CoV-2. It was first identified in December 2019 in Wuhan, Hubei, China, and has resulted in an ongoing pandemic. Common symptoms include fever, cough, fatigue, shortness of breath, and loss of smell and taste. While the majority of cases result in mild symptoms, some progress to ARDS. The time from exposure to onset of symptoms is typically around five days, but may range from two to fourteen days.

Thus, in some examples, the subject has a SARS-CoV-2 infection. In one example, the subject has COVID-19, for example, severe COVID-19. In particular, severe COVID-19 often results in ARDS. The methods of the present disclosure can be used to treat or prevent ARDS in a subject suffering from severe COVID-19.

In an example, the methods described herein comprise the identification of a subject having or suspected of having SARS-CoV-2. In this example, the subject may have one or more of the above symptoms and may be classified as having mild or severe SARS-CoV-2.

In an example, a method or use described herein further comprises a step of identifying a subject having or suspected of having mild COVID-19 based on a SARS-CoV-2 positive RT-PCR or molecular test result, and one or more of the following symptoms:

- fever;
- sore throat;
- headache;
- muscle pain (myalgia);
- gastrointestinal symptoms;
- cough;
- chest congestion;
- runny nose;
- wheezing;
- skin rash;
- eye irritation or discharge;
- chills;
- new or changing olfactory or taste disorders;
- red or bruised looking feet or toes;
- shaking chills or rigors;
- malaise (loss of appetite, generally unwell, fatigue, physical weakness).

In another example, a method or use described herein further comprises a step of identifying a subject having or suspected of having moderate COVID-19 based on a SARS-CoV-2 positive RT-PCR or molecular test result, and any one of the following new or worsening signs or symptoms:

- respiratory rate $2 \geq 20$ breaths/minute;
- abnormal saturation of oxygen but still $\geq 93\%$ on room air at sea level;
- clinical or radiologic evidence of pneumonia;
- radiologic evidence of DVT;
- shortness of breath or difficulty breathing;

or any two of the following new or worsening signs or symptoms:

- fever;
- heart rate $2 \geq 90$ beats/minute;
- shaking chills or rigors;
- new or changing olfactory or taste disorders;
- sore throat;
- malaise;

- headache;
- cough;
- muscle pain (myalgia);
- gastrointestinal symptoms;
- red or bruised looking feet or toes.

In another example, a method or use described herein further comprises a step of identifying a subject having or suspected of having severe COVID-19 based on a SARS-CoV-2 positive RT-PCR or molecular test result; and any one or more of the following:

- clinical signs at rest indicative of severe systemic illness (respiratory rate ≥ 30 breaths/minute, heart rate ≥ 125 beats/minute, $\text{SpO}_2 \leq 93\%$ on room air at sea level, or $\text{PaO}_2/\text{FiO}_2 \leq 300$ mmHg);
- respiratory failure (defined as needing high-flow oxygen, non-invasive ventilation, mechanical ventilation, or extracorporeal membrane oxygenation)
- evidence of shock (defined as systolic blood pressure ≤ 90 mmHg, diastolic blood pressure < 60 mmHg, or requiring vasopressors);
- significant acute renal, hepatic, or neurologic dysfunction;
- admission to the intensive care unit;
- death.

Acute Respiratory Distress Syndrome (ARDS)

The present disclosure provides, for example, methods of treating or preventing ARDS in a subject.

ARDS is a life-threatening condition characterized by bilateral pulmonary infiltrates, severe hypoxemia, and disruption of the alveolar-capillary membrane barrier (i.e., pulmonary vascular leak), leading to non-cardiogenic pulmonary edema. There is currently no effective pharmacological therapy.

Infectious etiologies, including influenza and coronavirus infection, are leading causes of ARDS. Accordingly, in one example of the present disclosure, the ARDS is associated with an influenza or a coronavirus infection. For example, the ARDS is associated with influenza. In another example, the ARDS is associated with a coronavirus infection, such as a SARS-COV infection. In one example, the ARDS is associated with a SARS-CoV-2 infection.

ARDS is classified according to the Berlin Definition, which includes:

- (1) presentation within 1 week of clinical insult or onset of respiratory symptoms;
- (2) acute hypoxemic respiratory failure, as determined by a $\text{PaO}_2/\text{FiO}_2$ ratio of 300 mmHg or less on at least 5 cm of continuous positive airway pressure (CPAP) or positive end expiratory pressure (PEEP), where PaO_2 is the partial pressure of oxygen in arterial blood and the FiO_2 is the fraction of inspired oxygen;

(3) bilateral opacities on lung radiographs not fully explained by effusions, consolidation, or atelectasis; and

(4) edema/respiratory failure not fully explained by cardiac failure or fluid overload.

In one example, the subject has or suffers from ARDS (i.e., the subject satisfies the Berlin definition of ARDS). For example, the subject is in need of treatment (i.e., in need thereof).

In one example, the subject has or suffers from a symptom associated with ARDS. Symptoms associated with ARDS and methods of identifying subjects at risk of developing ARDS will be apparent to the skilled person and/or are described herein. For example, the subject has one or more or all of the following symptoms:

- a) a respiratory frequency of greater than 30 breaths per minute;
- b) an oxygen saturation (SpO_2) of 93% or less on room air;
- c) a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) of less than 300 mmHg;
- d) a $\text{SpO}_2/\text{FiO}_2$ ratio of less than 218; and
- e) radiographic lung infiltrates in an amount of greater than 50%.

Currently, ARDS is classified as mild, moderate or severe with an associated increased mortality. The severity of ARDS can be categorized according to the Berlin definition as follows:

- (i) Mild ARDS: $\text{PaO}_2/\text{FiO}_2$ of 200-300 mmHg on at least 5 cm CPAP or PEEP;
- (ii) Moderate ARDS: $\text{PaO}_2/\text{FiO}_2$ of 100-200 mmHg on at least 5 cm PEEP; and
- (iii) Severe ARDS: $\text{PaO}_2/\text{FiO}_2$ of less than or equal to 100 mmHg on at least 5 cm PEEP.

In one example, the ARDS is mild ARDS. In another example, the ARDS is moderate ARDS. In a further example, the ARDS is severe ARDS.

In an example, the methods described herein comprise the identification of a subject having or suspected of having ARDS. In this example, the subject may have one or more of the above symptoms and may be classified as having mild or severe ARDS.

The methods of the present disclosure can, in addition to treatment of existing ARDS, be used to prevent the onset of ARDS. Thus, in one example, the subject does not have ARDS.

Respiratory Syncytial Virus (RSV)

The present disclosure provides, for example, methods of treating or preventing or delaying progress of RSV. RSV is an enveloped non-segmented negative-strand RNA virus in the family Paramyxoviridae, genus *Pneumovirus*. To infect a host cell, paramyxoviruses such as RSV, like other enveloped viruses such as influenza virus, require fusion of the viral membrane with a host cell's membrane.

In one example, the subject has or suffers from a symptom associated with RSV. Symptoms associated with RSV and methods of identifying subjects at risk of developing RSV will be apparent to the skilled person and/or are described herein. For example, the subject has one or more or all of the following symptoms indicative of mild RSV:

- a) congested or runny nose;
- b) dry cough;
- c) low-grade fever;
- d) sore throat;
- e) sneezing;
- f) headache;

or in severe cases:

- a) short, shallow and rapid breathing;
- b) struggling to breathe i.e., chest muscles and skin pull inward with each breath;
- c) cough;
- d) poor feeding;
- e) unusual tiredness (lethargy);
- f) irritability.

Thus, in one example, the RSV is mild RSV. In a further example, the RSV is severe RSV.

In an example, the methods described herein comprise the identification of a subject having or suspected of having RSV. In this example, the subject may have one or more of the above symptoms and may be classified as having mild or severe RSV.

The methods of the present disclosure can, in addition to treatment of existing RSV, be used to prevent the onset of RSV. Thus, in one example, the subject does not have RSV.

Kits

Another example of the disclosure provides kits containing a composition or vaccine of the present disclosure useful for the treatment or prevention of a disease or disorder as described above (e.g., a SARS-CoV-2 infection, COVID-19, RSV, influenza, ARDS).

In one example, the kit comprises a composition or vaccine of the present disclosure, optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions for use in treating or preventing or delaying progression of an infection in a subject in need thereof. In an example, composition or vaccine comprises a VLP comprising a fusion capsid protein and optionally an adjuvant such as MF59. In another example, the kit further comprises a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions to administer the composition or vaccine to a subject who is suffering from or at risk of suffering from a viral infection such as a SARS-CoV-2 infection, COVID-19, RSV, influenza.

Thus, in one example, the kit comprises:

- (a) a VLP disclosed herein, a pharmaceutical composition disclosed herein or a vaccine disclosed herein;
- (b) instructions for use thereof; and optionally
- (c) a pharmaceutically acceptable carrier, excipient or diluent.

In accordance with this example of the disclosure, the package insert is on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for a disease or disorder of the disclosure and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the VLP. The label or package insert indicates that the composition is used for treating a subject eligible for treatment, e.g., one having or predisposed to developing SARS-CoV-2 infection, RSV, influenza and/or ARDS or pneumonia in a subject having COVID-19, with specific guidance regarding dosing amounts and intervals of treatment and any other medicament being provided. The kit may further comprise an additional container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution, and/or dextrose solution. The kit may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

The present disclosure includes the following non-limiting Examples.

EXAMPLES

Example 1: Generation of VLPs

VLPs for use in the disclosure described herein can be synthesized through the individual expression of viral structural proteins, which can then self-assemble into the virus-like structure. Plasmid DNA encoding a VLP of the disclosure can be produced by inserting the polynucleotide sequence encoding a fusion capsid protein into a DNA vector. Suitable DNA vectors for use will be apparent to the skilled person. Insertion of the nucleotide sequence(s) into the DNA vector may be performed using standard methods in the art.

Briefly, competent bacterial cells (e.g., *Escherichia coli*) cells can be transformed with a DNA plasmid encoding a fusion capsid protein described herein. Individual bacterial colonies are isolated and the resultant plasmid DNA amplified in *E. coli* cultures. The plasmid DNA can be isolated following fermentation. For example, the plasmid DNA can be isolated using a commercially available kit (e.g., Maxiprep DNA kit), or other routine methods known to the skilled person. Following isolation, plasmid DNA can be linearized by restriction digest (i.e., using a restricting enzyme). Restriction enzymes are removed using methods known in the art, including for example phenol/chloroform extraction and ethanol precipitation.

The production of VLPs for use as a vaccine in accordance with the disclosure can be achieved by cell-based expression of the individual components to make up the VLP. For example, expression of viral structural proteins, which can then self-assemble into the virus-like structure can be achieved in HEK-293T or CHO cells. In this scenario, the VLPs lack infectious machinery and express the relevant TM protein domain and antigen protein, optionally bound to the capsid protein by a suitable linker.

VLPs generated in accordance with the disclosure are outlined in Figure 2 A-C and include:

1. AMV004 which comprises a capsid protein from Alfalfa Mosaic Virus (AMV), a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide. Various control VLP constructs may also be generated in accordance with the disclosure such as AMV001, AMV002 and AMV003;

2. AP003 which comprises a capsid protein from bacteriophage AP205, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide;

3. AP007 which comprises a capsid protein dimer from bacteriophage AP205, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide. Various control VLP constructs may also be generated in accordance with the disclosure such as AP001 and APV006;

4. MS003 which comprises a capsid protein from bacteriophage MS2, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide; and

5. MS007 which comprises a capsid protein dimer from bacteriophage MS2, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide. Various control VLP constructs may also be generated in accordance with the disclosure such as MS001 and MS006.

In any of the capsid fusion proteins listed above, the TM domain and antigen may be linked to the capsid protein by a linker amino acid sequence and may include a tag for detection such as a His6 tag.

Example 2: Effect of VLPs on immunogenicity *in vitro*

The effect of the VLPs outlined in Example 1 can be tested for immunogenicity *in vitro* by assessing read-outs such as LV microneutralisation, PV microneutralisation and ACE-2 binding inhibition. The effect of the VLPs can also be tested in the presence of an adjuvant such as MF-59.

In more detail, the VLPs can be assessed for antibody responses using a microneutralisation assay such as a short form assay (e.g., a virus fluorescent focus-based microneutralization assay). In another example, the microneutralization assay is a long form assay. The VLP can also be assessed for antibody responses. For example, the VLP can be assessed using a hemagglutination inhibition (HAI) assay. Methods of performing a HAI assay will be apparent to the skilled person and/or described, for example, in WHO (2011) *Manual for the laboratory diagnosis and virological surveillance of influenza*: WHO Press, World Health Organization.

The VLP can also be assessed for its ability to induce antigen specific T cell responses. Methods of assessing induction of antigen specific T cell responses will be apparent to the skilled person and/or are described herein. For example, antigen-specific T cell detection can be

performed on splenic cultures. Briefly, splenocyte cultures are established in T cell medium and cell cultures can either be stimulated with antigenic peptides or unstimulated. In one example, antigen-specific T cell responses can be determined using flow cytometry.

Example 3: VLP expression results

VLPs were generated using the methods described in Example 1. The constructs described in this Example are shown schematically in Figures 3 to 5 these include:

- A. AMV001 which comprises a capsid protein from Alfalfa Mosaic Virus (AMV), and a signal peptide;
- B. AMV002 which comprises a capsid protein from AMV, and a polyhistidine tag of 6 residues;
- C. AMV003 which comprises a capsid protein from AMV, a signal peptide, and a polyhistidine tag of 6 residues;
- D. AMV004 which comprises a capsid protein from AMV, a Spike (S) protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide;
- E. AMV005 which comprises a capsid protein from AMV, an S protein from a SARS-CoV-2 without a TM protein domain, and a signal peptide;
- F. AMV006 which comprises a capsid protein from AMV, and an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen;
- G. AMV007 which comprises a capsid protein from AMV, and an S protein from a SARS-CoV-2 without a TM protein domain;
- H. AMV008 which comprises a capsid protein from AMV, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen comprising a mutation at amino acid position 614 (D614G), a signal peptide, and a polyhistidine tag of 6 residues;
- I. AP001 which comprises a capsid protein from bacteriophage AP205, and a polyhistidine tag of 6 residues;
- J. AP003 which comprises a capsid protein from bacteriophage AP205, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a signal peptide;
- K. AP006 which comprises a capsid protein dimer from bacteriophage AP205, and a polyhistidine tag of 6 residues;
- L. AP007 which comprises a capsid protein dimer from bacteriophage AP205, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, a signal peptide, and a polyhistidine tag of 6 residues;

- M. AP008 which comprises a capsid protein from bacteriophage AP205, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, and a polyhistidine tag of 6 residues;
- N. MS001 which comprises a capsid protein from bacteriophage MS2, and a polyhistidine tag of 6 residues;
- O. MS003 which comprises a capsid protein from bacteriophage MS2, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, a signal peptide, and a polyhistidine tag of 6 residues;
- P. MS006 which comprises a capsid protein dimer from bacteriophage MS2, and a polyhistidine tag of 6 residues;
- Q. MS007 which comprises a capsid protein dimer from bacteriophage MS2, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, a signal peptide, and a polyhistidine tag of 6 residues;
- R. MS008 which comprises a capsid protein dimer from bacteriophage MS2, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, wherein the S protein lacks a furin cleavage site, a signal peptide, and a polyhistidine tag of 6 residues;
- S. MS009 which comprises a capsid protein dimer from bacteriophage MS2, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, a signal peptide, and a polyhistidine tag of 6 residues; and
- T. MS010 which comprises a capsid protein dimer from bacteriophage MS2, an S protein from a SARS-CoV-2 comprising a TM protein domain and an antigen, wherein the S protein lacks a furin cleavage site, a signal peptide, and a polyhistidine tag of 6 residues.

Amino acid sequences of all of the above constructs are provided in a table below.

Expression of several of the AMV VLP constructs was assessed in 293F and CHO cell lines. The results of these studies are summarised below in **Table 1**. Briefly, VLP expression was observed in 293F cells for constructs AMV002 and AMV005, while VLP expression was observed in CHO cells for constructs AMV001, AMV002, AMV003 and AMV005. These results indicate the VLPs could successfully be produced by recombinant expression and that CHO cells were more effective for the AMV constructs. It was also observed that the signal peptide sequence was not required for secretion of VLPs. Additionally, the AMV capsid protein can accommodate additions at the N- terminus.

Table 1. Summary expression results for several of the AMV constructs.

VLP	Capsid	N-terminal tag	C-terminal tag	Antigen?	Expression in 293F	Expression in CHO
-----	--------	----------------	----------------	----------	--------------------	-------------------

AMV001	AMV	Signal peptide	-	-	No	Yes
AMV002	AMV	-	His6x	-	Yes	Yes
AMV003	AMV	Signal peptide	His6x	-	No	Yes
AMV005	AMV	-	-	Spike (no TM)	Yes	Yes

Expression of several of the AP205 VLP constructs was assessed in 293F and CHO cell lines. The results of these studies are summarised below in **Table 2**. Briefly, VLP expression was observed in 293F cells for constructs AP001 and AP006, while VLP expression was observed in CHO cells for constructs AP001, AP003 and AP007. These results indicate that dimeric AP205 might allow for conjugation to large antigens. Additionally, having less antigen displayed appears to allow for more expression of these constructs. Importantly, AP205 has been used as a basis for RBD-VLP based vaccines.

Table 2. Summary of AP205 constructs and expression results.

VLP	Capsid	N-terminal tag	C-terminal tag	Antigen?	Expression in 293F	Expression in CHO
AP001	AP205	-	His6x	-	Yes	Yes
AP006	AP205 (dimer)	-	His6x	-	Yes	Yes
AP007	AP205 (dimer)	Signal peptide	His6x	D614G Spike	No	Yes

Expression of the MS2 VLP constructs was assessed in 293F and CHO cell lines. The results of these studies are summarised below in **Table 3**. Briefly, VLP expression was observed in 293F cells for constructs MS001, MS006, MS007, MS008, MS009 and MS010, while VLP expression was observed in CHO cells for all MS2 constructs. These results indicate that MS2 VLP constructs appear to be the most promising based on expression in these cell types and demonstrate that both monomeric and dimeric MS2 can display spike protein.

Table 3. Summary of MS2 constructs and expression results.

VLP	Capsid	N-terminal tag	C-terminal tag	Antigen?	Expression in 293F	Expression in CHO
MS001	MS2	-	His6x	-	Yes	Yes
MS003	MS2	Signal peptide	His6x	D614G Spike	No	Yes
MS006	MS2 (dimer)	-	His6x	-	Yes	Yes
MS007	MS2 (dimer)	Signal peptide	His6x	D614G Spike	Yes	Yes

MS008	MS2 (dimer)	Signal peptide	His6x	D614G Spike (furin mutation)	Yes	Yes
MS009	MS2 (dimer) no link	-	His6x	-	Yes	Yes
MS010	MS2 (dimer) no link	Signal peptide	His6x	D614G Spike (furin mutation)	Yes	Yes

Figure 6 shows an electron micrograph of the recombinantly expressed VLPs.

EXEMPLARY SEQUENCES OF THE DISCLOSURE

No.	Name	Sequence
1	AMV001	MFVFLVLLPLVSSMSSSQKKAGGKAGKPTKRSQNYAALRKAQL PKPPALKVPVVKPTNTILPQTGCVWQSLGTPLSLSSFNGLGVRFL YSFLKDFAGPRILEEDLIYRMVFSITPSYAGTFCLTDDVTTEDGR AVAHGNNPMQEFPHGAFHANEKFGFELVFTAPTHAGMQNQNFK HSYAVALCLDFDAQPEGSKNPSYRFNEVWVERKAFPRAGPLRS LITVGLLDEADDLDRH
2	AMV002	MSSSQKKAGGKAGKPTKRSQNYAALRKAQLPKPPALKVPVVKP TNTILPQTGCVWQSLGTPLSLSSFNGLGVRFLYSFLKDFAGPRIL EEDLIYRMVFSITPSYAGTFCLTDDVTTEDGRAVAHGNNPMQEF HGAFHANEKFGFELVFTAPTHAGMQNQNFKHSYAVALCLDFD AQPEGSKNPSYRFNEVWVERKAFPRAGPLRSLITVGLLDEADDL DRHGSSHHHHHH
3	AMV003	MFVFLVLLPLVSSMSSSQKKAGGKAGKPTKRSQNYAALRKAQL PKPPALKVPVVKPTNTILPQTGCVWQSLGTPLSLSSFNGLGVRFL YSFLKDFAGPRILEEDLIYRMVFSITPSYAGTFCLTDDVTTEDGR AVAHGNNPMQEFPHGAFHANEKFGFELVFTAPTHAGMQNQNFK HSYAVALCLDFDAQPEGSKNPSYRFNEVWVERKAFPRAGPLRS LITVGLLDEADDLDRHGSSHHHHHH
4	AMV004	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFHVISGTNGTKRFDNPVLPFNDGV YFASIEKSNIIRGWIFGTTLDSKTQSLLIVNNATNVVIKVCEFQFC NDPFLDHKNKNSWMESEFRVYSSANNCTFEYVSQPFLMDLEGK QGNFKNLREFVFKNIDGYFKIYSKHTPIIVREPEDLPQGFSALEPL VDLPIGINITRFQTLALHRSYLTSGDSSSGWTAGAAAYVGYL QPRTFLLKYNENGTITDAVDCALDPLSEKCTLKSTVEKGIYQT SNFRVQPTESIVRFPNITNLCPFDEVFNATRFASVYAWNKRISN CVADYSVLYNLAPFFTFKCYGVSPKLNLDLCFTNVYADSFVIRG DEVQRQIAPGQTGNIADYNYKLDDFTGCVIAWNSNKLDSKVS NINYLYRLFRKSNLKPFRDISTEIQAGNKPNGVAGFNCYFP LRSYSFRPTYGVGHQPYRVVLSFELLHAPATVCGPKKSTNLVK NKCYNFNFNGLKGTGVLTESNKKFLPFQFGRDIADTTDAVRDP QTLEILDITPCSFSGVSVITPGTNTSNQVAVLYQGVNCTEVPVAI HADQLTPTWRVYSTGSNVFQTRAGCLIGA EYVNNSYECDIPIGA GICASYQTQTKSHGSASSVASQSIAYTMSLGAENSVAYSNNISAI

No.	Name	Sequence
		PTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFC TQLKRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKYFGGFNFSQI LPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLI CAQKFKGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSL SSTASALGKLQDVVNHNAAQALNTLVKQLSSKFGAISSVLNDIFS RLDPPAEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAAT KMSECVLGQSKRVDFCGKGYHLSFPQSAPHGVVFLHVTYVP AQEKNFTTAPAICHGDKAHFPREGVFVSNGTHWFVTQRNFYEP QIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFK NHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQE LGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKG CCSCGSCCKFDEDDSEPVLKGVKLHYTGGGGSGGGGSGGGGS MSSSQKKAGGKAGKPTKRSQNYAALRKAQLPKPPALKVPVVKP TNTILPQTGCVWQSLGTPLSLSSFNGLGVRFLYSLKDFAGPRIL EEDLIYRMVFSITPSYAGTFCLTDDVTTEDGRAVAHGNPMQEF HGAFHANEKFGFELVFTAPTHAGMQNQNFKHSYAVALCLDFD AQPEGSKNPSYRFNEVWVERKAFPRAGPLRSLITVGLLDEADDL DRH
5	AMV005	MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFHVISGTNGTKRFDNPVLPFNDGV YFASIEKSNIIRGWIFGTTLDSTQSLIVNNATNVVIKVFCEQFC NDPFLDHKNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGK QGNFKNLREFVFKNIDGYFKIYSKHTPIIVREPEDLPQGFSALEPL VDLPIGINITRFQTLALHRSYLT PGDSSSGWTAGAAAYVGYL QPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSTVEKGIYQT SNFRVQPTESIVRFPNITNLCPFDEVFNATRFASVYAWNKRISN CVADYSVLYNLAPFFTFKCYGVSPTKLNDLCFTNVYADSFVIRG DEVRQIAPGQTGNIADYNYKL PDDFTGCVIAWNSNKLDSKVS G NYNYLYRLFRKSNLKPFERDISTEIQAGNKPNGVAGFNCYFP LRSYSFRPTYGVGHQPYRVVLSFELLHAPATVCGPKKSTNLVK NKC NVNFNGLKGTGVLTESNKKFLPFQQFGRDIADTTDAVRDP QTLEILDITPCSFGGVS VITPGTNTSNQVAVLYQGVNCTEVPVAI HADQLTPTWRVYSTGSNVFQTRAGCLIGAEYVNNSECDIPIGA GICASYQTQTKSHGSASSVASQSIIAYTMSLGAENSVAYSNNIAI PTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFC TQLKRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKYFGGFNFSQI LPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLI CAQKFKGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSL SSTASALGKLQDVVNHNAAQALNTLVKQLSSKFGAISSVLNDIFS RLDPPAEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAAT KMSECVLGQSKRVDFCGKGYHLSFPQSAPHGVVFLHVTYVP AQEKNFTTAPAICHGDKAHFPREGVFVSNGTHWFVTQRNFYEP QIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFK NHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQE LGKYEQYIKGGGGSGGGGSGGGGSMSSSQKKAGGKAGKPTKR SQNYAALRKAQLPKPPALKVPVVKPTNTILPQTGCVWQSLGTPL SLSSFNGLGVRFLYSLKDFAGPRILEEDLIYRMVFSITPSYAGTF CLTDDVTTEDGRAVAHGNPMQEFPHGAFHANEKFGFELVFTAP THAGMQNQNFKHSYAVALCLDFDAQPEGSKNPSYRFNEVWVE RKAFFRAGPLRSLITVGLLDEADDLDRH

No.	Name	Sequence
6	AMV006	MQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFL PFFSNVTWFHVISGTNGTKRFDNPVLPFNDGVYFASIEKSNIIRG WIFGTTLDSKTQSLIVNNATNVVIKVEFCNDPFLDHKNNK SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFV FKNIDGYFKIYSKHTPIIVREPEDLPQGFSALEPLVDLPIGINITRFQ TLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNEN GTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIV RFPNITNLCPFDEVFNATRFASVYAWNRKRISNCVADYSVLYNL APFFTFKCYGVSPTKLNLCFTNVYADSFVIRGDEVQRQIAPGQT GNIADYNYKLPDDFTGCVIAWNSNKLDSKVSGNYNYLYRLFRK SNLKPFERDISTEIYQAGNKPCNGVAGFNCYFPLRSYSFRPTYGV GHQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFGL KGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCS FGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWR VYSTGSNVFQTRAGCLIGA EYVNNSYECDIPIGAGICASYQTQTK SHGSASSVASQSIIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEI LPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLKRALTGI AVEQDKNTQEVFAQVKQIYKTPPIKYFGGFNFSQILPDPSKPSKR SFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLICAQKFKGLTV LPPLLTDemiaQYTSALLAGTITSGWTFGAGAAALQIPFAMQMAY RFNGIGVTQNVLYENQKLIANQFN SAIGKIQDSLSTASALGKLQ DVVNHNAQALNTLVKQLSSKFGAISSVLNDIFSRLDPPEAEVQID RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSK RVDFCGKGYHLMSPQSAPHGVVFLHVTVVPAQEKNFTTAPAI CHDGKAHFPREGVFSNGTHWFVTQRNFYEPQIITTDNTFVSGN CDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDIS GINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPW YIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDED DSEPVLKGVKLHYTGGGGSGGGGSGGGGSMSSQKKAGGKA GKPTKRSQNYAALRKAQLPKPPALKVPVVKPTNTILPQTGCVW QSLGTPLSLSSFNGLGVRFLYSFLKDFAGPRILEEDLIYRMVFSIT PSYAGTFCLTDDVTTEDEGRAVAHGPNMQEFPHGAFHANEKFGF ELVFTAPTHAGMQNQNFKHSYAVALCLDFDAQPEGSKNPSYRF NEVWVERKAFFRAGPLRSLITVGLLDEADDLDRH
7	AMV007	MQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFL PFFSNVTWFHVISGTNGTKRFDNPVLPFNDGVYFASIEKSNIIRG WIFGTTLDSKTQSLIVNNATNVVIKVEFCNDPFLDHKNNK SWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFV FKNIDGYFKIYSKHTPIIVREPEDLPQGFSALEPLVDLPIGINITRFQ TLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNEN GTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIV RFPNITNLCPFDEVFNATRFASVYAWNRKRISNCVADYSVLYNL APFFTFKCYGVSPTKLNLCFTNVYADSFVIRGDEVQRQIAPGQT GNIADYNYKLPDDFTGCVIAWNSNKLDSKVSGNYNYLYRLFRK SNLKPFERDISTEIYQAGNKPCNGVAGFNCYFPLRSYSFRPTYGV GHQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFNFGL KGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCS FGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWR VYSTGSNVFQTRAGCLIGA EYVNNSYECDIPIGAGICASYQTQTK SHGSASSVASQSIIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEI LPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLKRALTGI AVEQDKNTQEVFAQVKQIYKTPPIKYFGGFNFSQILPDPSKPSKR SFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLICAQKFKGLTV

No.	Name	Sequence
		LPPLLTDemiaQYTSALLAGTITSGWTFGAGAALQIPFAMQMAY RFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQ DVVNHNAQALNTLVKQLSSKFGAISSVLNDIFSRLDPPEAEVQID RLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSK RVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFHTTAPAI CHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSGN CDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLDGDIS GINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKGGG GSGGGGSGGGGSMSSSQKKAGGKAGKPTKRSQNYAALRKAQ LPKPPALKVPVVKPTNTILPQTGCVWQSLGTPLSLSSFNGLGVRF LYSFLKDFAGPRILEEDLIYRMVFSITPSYAGTFCLTDDVTTEDG RAVAHGNPMQEFPHGAFHANEKFGFELVFTAPTHAGMQNQNF KHSY AVALCLDFDAQPEGSKNPSYRFNEVWVERKAFPRAGPLR SLITVGLLDEADDLDRH
8	AMV008	MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFHVISGTNGTKRFDNPVLPFNDGV YFASIEKSNIRGWIFGTTLDSTQSLIVNNAATNVVKEFQFC NDPFLDHKNNKSWMESEFRVYSSANNCTFEYVSQPFMLDLEGK QGNFKNLREFVFKNIDGYFKIYSKHTPIIVREPDLPGQFSALEPL VDLPIGINITRFQTLALHRSYLTPGDSSSGWTAGAAAYYVGYL QPRTFLLKYNENGTTDAVDCALDPLSEKCTLKSTVEKGIYQT SNFRVQPTESIVRFPNITNLCPFDEVFNATRFASVYAWNKRISN CVADYSVLYNLAPFFTFKCYGVSPTKLNDLCFTNVYADSFVIRG DEVRQIAPGQTGNIADYNYKLDDFTGCVIAWNSNKLDSKVSG NYNYLYRLFRKSNLKPFRDISTEIYQAGNKPNGVAGFNCYFP LRSYSFRPTYGVGHQPYRVVLSFELLHAPATVCGPKKSTNLVK NKCVMNFNGLKGTGVLTESNKKFLPFQQFGRDIADTTDAVRDP QTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAI HADQLTPTWRVYSTGSNVFQTRAGCLIGAEYVNNSECDIPIGA GICASYQTQTKSHGSASSVASQSIAYTMSLGAENSVAYSNNIAI PTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLQYGSFC TQLKRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKYFGGFNSQI LPDSPKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI CAQKFKGLTVLPLLTDemiaQYTSALLAGTITSGWTFGAGAAL QIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSL SSTASALGKLQDVVNHNAQALNTLVKQLSSKFGAISSVLNDIFS RLDPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAAT KMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVP AQEKNFHTTAPAI CHDGKAHFPREGVFVSNGTHWFVTQRNFYEP QIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFK NHTSPDVLDGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQE LGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKG CCSCGSCCKFDEDDSEPVLKGVKLHYTGGGGSGGGGSGGGGS MSSSQKKAGGKAGKPTKRSQNYAALRKAQLPKPPALKVPVVKP TNLTPQTGCVWQSLGTPLSLSSFNGLGVRFLYSFLKDFAGPRIL EEDLIYRMVFSITPSYAGTFCLTDDVTTEDGRAVAHGNPMQEF HGAFHANEKFGFELVFTAPTHAGMQNQNFKHSY AVALCLDFD AQPEGSKNPSYRFNEVWVERKAFPRAGPLRSLITVGLLDEADDL DRHGSSHHHHHH
9	AP001	MANKPMQPITSTANKIVWSDPTLSTTFASLLRQRVKVGIAEL NNVSGQYVSVYKRPAPKEGCADACVIMPNNQSIRTVISGSAE NLATLKAEWETHKRNVDTLFASGNAGLGLDPTAAIVSSDTTA GSSHHHHHH

No.	Name	Sequence
10	AP003	MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFND GVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNAATNVVIKVC QFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFL MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGF SALEPLVDLPIGINITRFQTLALHRSYLTSGDSSSGWTAGAAAY YVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLSFTVE KGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWN RKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNLCFTNVYAD SFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNLD SKVGGNYNYLYRLFRKSNLKPFERDISTEYIYQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVVLSEFLLHAPATVCGPKKS TNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTD AVRDPQTLEILDITPCSFSGVSVITPGTNTSNQVAVLYQGVNCTE VPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSEYEC DIPIGAGICASYQTQTNSPRRARSVASQSIAYTMSLGAENSVAYS NNSIAIPTNFTISVTTEILPVSMKTSVDCTMYICGDSSTECNLLL QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKIYKTPPIKDFG GFNFSQILPDPSKPSKRSFIEDLLFNKVTADAGFKIYQYGDCLGDI AARDLICAQKFNGLTVLPPLLDEMIAQYTSALLAGTITSGWTF GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAI GKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS VLNDILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA SANLAATKMSECVLGQSKRVDFCGKGYHLSMFPQSAPHGVVFL HVTYVPAQEKNTTAPAICHGDKAHFPREGVFSNGTHWVFTQ RNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEEL DKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESL IDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCC SCLKGCCSCGSCCKFDEDDSEPVKGVKLHYTGGSGGGGGSGM ANKPMQPITSTANKIVWSDPTLSTTFASLLRQRVKVGIAELNN VSGQYVSVYKRPAPKPEGCADACVIMPNNQSIRTVISGSAENL ATLKAETHKRNVDTLFASGNAGLGFLDPTAAIVSSDTTAGSS HHHHHH
11	AP006	MANKPMQPITSTANKIVWSDPTLSTTFASLLRQRVKVGIAEL NNVSGQYVSVYKRPAPKPEGCADACVIMPNNQSIRTVISGSAE NLATLKAETHKRNVDTLFASGNAGLGFLDPTAAIVSSDTTA GGGGGMANKPMQPITSTANKIVWSDPTLSTTFASLLRQRVKV GIAELNNVSGQYVSVYKRPAPKPEGCADACVIMPNNQSIRTVIS GSAENLATLKAETHKRNVDTLFASGNAGLGFLDPTAAIVSS DTTAGSSHHHHHH
12	AP007	MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFND GVYFASTEKSNIIRGWIFGTTLDSTQSLIVNNAATNVVIKVC QFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFL MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGF SALEPLVDLPIGINITRFQTLALHRSYLTSGDSSSGWTAGAAAY YVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLSFTVE KGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWN RKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNLCFTNVYAD SFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNLD SKVGGNYNYLYRLFRKSNLKPFERDISTEYIYQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVVLSEFLLHAPATVCGPKKS TNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTD

No.	Name	Sequence
		AVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTE VPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYEC DIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYS NNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECNLLL QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFG GFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTF GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAI GKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS VLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA SANLAATKMSECVLGQSKRVDFCGKGYHLSMFPQSAPHGVVFL HVTYVPAQEKNFTTAPAICHGDKAHFPREGVFVSNGTHWFTVQ RNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEEL DKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESL IDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCC SCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYTGSGGGGGSGSM ANKPMQPITSTANKIVWSDPTRLSTTFASLLRQRVKVGIAELNN VSGQYVSVYKRPAPKPEGCADACVIMPENQSIIRTVISGAENL ATLKAEWETHKRNVDTLFASGNAGLGFLDPTAAIVSSDTTAGG GGMANKPMQPITSTANKIVWSDPTRLSTTFASLLRQRVKVGI AELNNVSGQYVSVYKRPAPKPEGCADACVIMPENQSIIRTVISG SAENLATLKAEWETHKRNVDTLFASGNAGLGFLDPTAAIVSSDT TAGSSHHHHHH
13	AP008	MQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFL PFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYFASTEKSNII RGWIFGTTLDSKTQSLIIVNNATNVVIKVCEQFCNDPFLGVY HKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFK NLREFVFKNIDGYFKIYSKHTPINLVRDLPPQGSFALEPLVDLPIGI NITRFQTLALHRSYLTTPGDSSSGWTAGAAAYVGYLQPRFTLL KYNENGITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQ PTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRKISNCVADYS VLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVYRQI APGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYL YRLFRKSNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVN FNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEIL DITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQL TPTWRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASY QTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNNSIAIPTNFTI SVTTEILPVSMTKTSVDCTMYICGDSTECNLLLQYGSFCTQLNR ALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPS KPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLICAQKF NGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFA MQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTAS ALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDKV EAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSEC VLGQSKRVDFCGKGYHLSMFPQSAPHGVVFLHVTYVPAQEKNF TTAPAICHGDKAHFPREGVFVSNGTHWFTVQRNFYEPQIITTDN TFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQ YIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGS CCKFDEDDSEPVLKGVKLHYTGSGGGGGSGGGSGGGGGSGM ANKPMQPITSTANKIVWSDPTRLSTTFASLLRQRVKVGIAELNN

No.	Name	Sequence
		VSGQYVSVYKRPAPKPEGCADACVIMPNENQSIRTVISGSAENL ATLKAEWETHKRNVDTLFASGNAGLGFLDPTAAIVSSDTTAGG GGGMANKPMQIPITSTANKIVWSDPTLSTTFASALLRQRVKVGI AELNNVSGQYVSVYKRPAPKPEGCADACVIMPNENQSIRTVISG SAENLATLKAEWETHKRNVDTLFASGNAGLGFLDPTAAIVSSDT TAGSSHHHHHH
14	MS001	MASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAY KVTCsvRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSY LNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANS GIYGS SHHHHHH
15	MS003	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFND GVYFASTEKSNIIRGWIFGTTLDSKTQSLIVN NATNVVIK VCEF QFCNDPFLGVYHKNKSWMESEFRVYSSANNCTFEYVSQPFL MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDL PQGF SALEPLVDLPIGINITRFQTLALHRSYLT PGDSSSGW TAGAAAY YVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVE KGIYQTSNFRVQPTESIVRFPNITNLC PFGEVFNATRFASVYAWN RKRI SN CVADYSVLYNSASFSTFKCYGVSP TKLNDLCFTNVYAD SFVIRGDEV RQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLD SKVGGNYNYLYRLFRKSNLKPFERDISTEYIYQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVVL SFELLHAPATVCGPKKS TNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTD AVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTE VPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGA EHVNN SYEC DIPIGAGICASYQTQTN SPRRARSV ASQSIIAYTMSLGAENSVAYS NNSIAIPTNFTISVTTEILPVSM TKTSVDCTMYICGDSTEC SNLLL QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFG GFNFSQLPDPSPKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPLL TDEMIAQYTSALLAGTITSGWTF GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFN SAI GKIQDSL SSTASALGKLQDVVNQNAQALNTLVKQLSSNF GAISS VLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAEIRA SANLAATKMSECVLGQSKRVDFCGKGYHLM SFPQSAPHGVVFL HVTYVPAQEKNFTTAPAICH DGKAHFPREGVFVSNGTHW FVTQ RNFYEPQIITTDNTFVSGNCDV VIGIVNNTVYDPLQPELDSFKEEL DKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESL IDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCMTSCC SCLKGCCSCGSCCKFDEDDSEPVLKG VKLHYTGGSGGGGGSGM ASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAYK VTCSVRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSYL NMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANS GIYGS HHHHHH
16	MS006	MASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAY KVTCsvRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSY LNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANS GIYGG GGGMASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRS QAYKVTCsvRQSSAQNRKYTIKVEVPKVATQTVGGVELPVA A WRSY LNMELTIPIFATNSDCELIVKAMQGLLKDGNPIPSAIAANS GIYGS SHHHHHH
17	MS007	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFND GVYFASTEKSNIIRGWIFGTTLDSKTQSLIVN NATNVVIK VCEF

No.	Name	Sequence
		QFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFL MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGF SALEPLVDLPIGINITRFQTLALHRSYLTGDDSSSGWTAGAAAY YVGYLQPRTFLLKYNENGTITDAVDCALDPLSEKCTLKSFTVE KGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWN RKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNLDLCTNRYAD SFVIRGDEVRIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLD SKVGGNYNYLYRLFRKSNLKPFERDISTEYQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKS TNLVKNKCVNFNENGLTGTGVLTESNKKFLPFQQFGRDIADTTD AVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTE VPVAIHADQLTPTWRVYSTGNSVFQTRAGCLIGAETHVNNSEYEC DPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYS NNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSSTECNLLL QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFG GFNFSQILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTF GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAI GKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS VLNDILSRDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA SANLAATKMSECVLGQSKRVDFCGKGYHLSFPPQSAPHGVVFL HVTYVPAQEKNTTAPAICHGDKAHFPREGVFSNGTHWFTVQ RNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEEL DKYFKNHTSPDVLGDISGINASVNIQKEIDRLNEVAKNLNESL IDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCC SCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYTGGSGGGGGSGM ASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNRSQAYK VTCVSRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSYL NMELTIPIFATNSDCELVKAMQGLLKDGNPIPSAIAANSIYGG GGGMASNFTQFVLVDNGGTGDVTVAPSNFANGVAEWISSNRS QAYKVTCVSRQSSAQNRKYTIKVEVPKVATQTVGGVELPVA WRSYLNMELTPIFATNSDCELVKAMQGLLKDGNPIPSAIAANS GIYGSSSHIIHHH
18	MS008	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFAIHVSGTNGTKRFDNPVLPFND GVYFASTEKSNIIRGWIFGTTLDSTQSLNINATNVVIKVCFF QFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFL MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGF SALEPLVDLPIGINITRFQTLALHRSYLTGDDSSSGWTAGAAAY YVGYLQPRTFLLKYNENGTITDAVDCALDPLSEKCTLKSFTVE KGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWN RKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNLDLCTNRYAD SFVIRGDEVRIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLD SKVGGNYNYLYRLFRKSNLKPFERDISTEYQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKS TNLVKNKCVNFNENGLTGTGVLTESNKKFLPFQQFGRDIADTTD AVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTE VPVAIHADQLTPTWRVYSTGNSVFQTRAGCLIGAETHVNNSEYEC DPIGAGICASYQTQTNSPGSASSVASQSIIAYTMSLGAENSVAYS NNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSSTECNLLL QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFG GFNFSQILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTF

No.	Name	Sequence
		GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAI GKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS VLNDILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQILIRAAEIRA SANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAHPHGVVFL HVTYVPAQEKNTTAPAICHGDKAHFPREGVFVSNGTHWFVTQ RNFYEPQIITDNTFVSGNCDVIGIVNNTVYDPLQPELDSFKEEL DKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESL IDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLGSGGGG GSGGGSGGGGSGMASNFTQFVLVDNNGGTGDVTVAPSNFANG VAEWISSNSRSQAYKVTCSVRQSSAQNRKYTIKVEVPKVATQTV GGVELPVAAWRSYLNMEITPIFATNSDCELVKAMQGLLKDGN PIPSAIAANSIYGGGGGSGMASNFTQFVLVDNNGGTGDVTVAPSN FANGVAEWISSNSRSQAYKVTCSVRQSSAQNRKYTIKVEVPKV ATQTVGGVELPVAAWRSYLNMEITPIFATNSDCELVKAMQGL LKDGNPIPSAIAANSIYGGSSHHHHHH
19	MS009	MASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAY KVTCSVRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSY LNMEITPIFATNSDCELVKAMQGLLKDGNPIPSAIAANSIY ASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAY VTCSVRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSY LNMEITPIFATNSDCELVKAMQGLLKDGNPIPSAIAANSIYGGSS HHHHHH
20	MS010	MFVFLVLLPLVSSQCVNLTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFND GVYFASTEKSNIRGWIFGTTLDSKTQSLIVNNAITNVVIVKCEF QFCNDPFLGVYHKNKSWMESEFRVYSSANNCTFEYVSQPFL MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPOGF SALEPLVDLPIGINITRFQTLALHRSYLTGPDSSSGWTAGAAAY YVGYLQPRTELLKYNENGTITDAVDCALDPLSETKCTLKSFTVE KGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWN RKRISSNCVADYSVLYNSASFSTFKCYGVSPKLNLDLCTNVYAD SFVIRGDEVQRQIAPGGTGKIADYNYKLPDDFTGCVIANSNNLD SKVGGNYNYLYRLFRKSNLKPFERDISTEYQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVVLSEFLLHAPATVCGPKKS TNLVKNKCVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTD AVRDPQTEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTE VPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSEYEC DPIGAGICASYQTQTNSPGSASSVASQSIIAYTMSLGAENSVAYS NNSIAIPTNFTISVTTEILPVSMKTSVDCTMYICGDSSTECNLLL QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFG GFNFSQILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPLLDEMIAQYTSALLAGTITSGWTF GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAI GKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS VLNDILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQILIRAAEIRA SANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAHPHGVVFL HVTYVPAQEKNTTAPAICHGDKAHFPREGVFVSNGTHWFVTQ RNFYEPQIITDNTFVSGNCDVIGIVNNTVYDPLQPELDSFKEEL DKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESL IDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLGSGGGG GSGGGSGGGGSGMASNFTQFVLVDNNGGTGDVTVAPSNFANG VAEWISSNSRSQAYKVTCSVRQSSAQNRKYTIKVEVPKVATQTV GGVELPVAAWRSYLNMEITPIFATNSDCELVKAMQGLLKDGN

No.	Name	Sequence
		PIPSAIAANSIGIYMASNFTQFVLVDNNGGTGDVTVAPSNFANGVA EWISSNSRSQAYKVTCVSRQSSAQNRKYTIKVEVPKVATQTVGG VELPVAAWRSYLNEMELTIPIFATNSDCELVKAMQGLLKDGNPIP SAIAANSIGIYGSSHHHHH
21	Signal peptide	MFVFLVLLPLVSS
22	AMV004 linker	GGSGGGGGSGGGSG
23	AMV005 linker	GGSGGGGGSGGGSGGGGGSG
24	AMV006 linker	GGSGGGGGSGGGSGGGGGSGGGSGGGGGSGGGSGGGGGSG
25	AP003/004/007 linker	GGSGGGGGSG
26	AP005/008 linker	GGSGGGGGSGGGSGGGGGSG
27	AP006/007 AP205 dimer linker	GGGGG
28	His6x tag	GSSHHHHH
29	SARS-CoV-2 S protein (wt, no signal peptide)	QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLP FFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIR GWIFGTTLDSKTQSLIVNNAATNVVIKVEFCFQCNDFPLGVYYH KNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNL REFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINIT RFQTLALHRSYLTSGDSSSGWTAGAAAYYVGYLQPRTFLLKY NENGTITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPT SIVRFPNITNLCPFGEVFNATRFASVYAWNKRKISNCVADYSVLY NSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQ TGKIADYNYKLDDFTGCVIAWNSNNLDSKVGNGYNYLYRLFR KSNLKPFRDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNG VGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNENFNG LTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDITPCS FGGVSIVTPTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWR VYSTGSNVFQTRAGCLIGAEHVNNSECDIPIGAGICASYQTQTN SPRRARSVASQSIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEI LPVSMTKTSVDCTMYICGDSTECNLLLQYGSFCTQLNRALTGI AVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSPKSKR SFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLCAQKFNGLTV LPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIPFAMQMAY RFNGIGVTQNVLYENQKLIANQFNSAIGKIQDLSSTASALGKLQ DVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDLKVEAEVQI DRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQS KRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFHTTAPA ICHDKAHFHPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSG NCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGD ISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWP WYIWLGFIAGLIAIVMTIMLCCMTSCCCLKGCCSCGSCCKFD EDDSEPVKGVKLHYT
30	SARS-CoV-2 S protein – wt, without TM domain	QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLP FFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIR GWIFGTTLDSKTQSLIVNNAATNVVIKVEFCFQCNDFPLGVYYH KNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNL REFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINIT RFQTLALHRSYLTSGDSSSGWTAGAAAYYVGYLQPRTFLLKY

No.	Name	Sequence
		NENGTITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRFLRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPACHDGGKAHFPREGVFVSNGTHWFTVQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIK
31	SARS-CoV-2 S protein with D614G mutation, relative to wt	QCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIRGWIFGTTLDSKTQSLIVNNATNVVIKVECFQCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFMDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLALHRSYLTTPGDSSSGWTAGAAAYYVGYLQPRFTLLKY NENGTITDAVDCALDPLSETKCTLSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRFLRKSNLKPFERDISTEIQAGSTPCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKCVNFFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLNDILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTAPACHDGGKAHFPREGVFVSNGTHWFTVQRNFYEPQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVKGVKLHYT
32	AMV capsid protein	MSSSQKKAGGKAGKPTKRSQNYAALRKAQLPKPPALKVPVVKPTNTILPQTGCVWQSLGTPLSLSSFNGLGVRFLYSLFKDFAGPRILEEDLIYRMVFSITPSYAGTFCLTDDVTTEDGRAVAHGNPMQEF

No.	Name	Sequence
		HGAFHANEKFGFELVFTAPTHAGMQNQNFKHSYAVALCLDFD AQPEGSKNPSYRFNEVWVERKAFPRAGPLRSLITVGLLDEADDL DRH
33	AP205 capsid protein	MANKPMQPITSTANKIVWSDPTRLSTTFASLLRQRVKVGIAEL NNVSGQYVSVYKRPAPKPEGCADACVIMPNNENQSIRTVISGSAE NLATLKAEWETHKRNVDTLFASGNAGLGFLDPTAAIVSSDTTA
34	AP205 capsid protein dimer	MANKPMQPITSTANKIVWSDPTRLSTTFASLLRQRVKVGIAEL NNVSGQYVSVYKRPAPKPEGCADACVIMPNNENQSIRTVISGSAE NLATLKAEWETHKRNVDTLFASGNAGLGFLDPTAAIVSSDTTA GGGGGMANKPMQPITSTANKIVWSDPTRLSTTFASLLRQRVK GIAELNNVSGQYVSVYKRPAPKPEGCADACVIMPNNENQSIRTVIS GSAENLATLKAEWETHKRNVDTLFASGNAGLGFLDPTAAIVSS DTTA
35	MS2 capsid protein	MASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAY KVTCSVRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSY LNMELTIPIFATNSDCELVKAMQGLLKDGNPIPSAIAANSIGY
36	MS2 capsid protein dimer with linker	MASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAY KVTCSVRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSY LNMELTIPIFATNSDCELVKAMQGLLKDGNPIPSAIAANSIGYGG GGGMASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRS QAYKVTCSVRQSSAQNRKYTIKVEVPKVATQTVGGVELPVA WRSYLNMELTIPIFATNSDCELVKAMQGLLKDGNPIPSAIAANS GIYG
37	MS2 capsid protein dimer without linker	MASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAY KVTCSVRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSY LNMELTIPIFATNSDCELVKAMQGLLKDGNPIPSAIAANSIGYM ASNFTQFVLVDNNGGTGDVTVAPSNFANGVAEWISSNSRSQAYK VTCSVRQSSAQNRKYTIKVEVPKVATQTVGGVELPVAAWRSY NMELTIPIFATNSDCELVKAMQGLLKDGNPIPSAIAANSIGY
38	Full length wt SARS-CoV-2 S protein (including signal peptide)	MFVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFR SSVLHSTQDLFLPFFSNVTWFWHAIHVSGTNGTKRFDNPVLPFND GVYFASTEKSNIIRGWIFGTTLDSTQSLNINATNVVIKVC QFCNDPFLGVYHKNKSWMESEFRVYSSANNCTFEYVSPFL MDLEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLRDLQGF SALEPLVDLPIGINITRFQTLALHRSYLTSGDSSSGWTAGAAAY YVGYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLSFTVE KGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWN RKRISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNVYAD SFVIRGDEVQRQIAPGQTGKIADYNYKLPDDFTGCVIAWNSNLD SKVGGNYNYLYRLFRKSNLKPFERDISTEIQAGSTPCNGVEGF NCYFPLQSYGFQPTNGVGYQPYRVVVLSEFLLHAPATVCGPKKS TNLVKNKCVNFNGLTGTGVLTESNKKFLPFQFGRDIADTTD AVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTE VPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAHEVNNSYEC DIPGAGICASYQTQTNPRRARSVASQSIIAYTMSLGAENSVAYS NNSIAIPTNFTISVTTEILPVSMTKTSVDCTMYICGDSSTECNLL QYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFG GFNFSQILPDPSKPSKRSFIEDLLFNKVTADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPLLTDEMIAQYTSALLAGTITSGWTF GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAI GKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS VLNDILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRA SANLAATKMSECVLGQSKRVDFCGKGYHLMSPQSAHPHGVVFL

No.	Name	Sequence
		HVTYVPAQEKNFTTAPAICHGDKAHFPREGVFVSNGTHWFTQ RNFYEPQIITDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEEL DKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESL IDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMVTIMLCCMTSCC SCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT

NUMBERED STATEMENTS OF THE DISCLOSURE

The present disclosure provides at least the following numbered statements:

1. A recombinant virus-like particle (VLP) comprising a capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:
 - (a) a capsid protein from a non-enveloped virus;
 - (b) a transmembrane (TM) protein domain; and
 - (c) an antigen protein,
 wherein the capsid protein is encapsulated within the lipid bilayer.
2. The recombinant VLP of statement 1, wherein the capsid protein from a non-enveloped virus is from an alfalfa mosaic virus (AMV), bacteriophage MS2 or bacteriophage AP205.
3. The recombinant VLP of statement 2, wherein the capsid protein is a dimer.
4. The recombinant VLP of any one of statements 1 to 3, wherein the capsid protein is fused to the TM protein domain by a peptide linker.
5. The recombinant VLP of any one of statements 1 to 4, wherein the TM protein domain and the antigen protein are from a virus.
6. The recombinant VLP of statement 5, wherein the TM protein domain and the antigen protein are from the same virus.
7. The recombinant VLP of statement 5, wherein the TM protein domain and the antigen protein are from different viruses.
8. The recombinant VLP of any one of statements 1 to 6, wherein the antigen protein and the TM protein domain are from a SARS-CoV-2.
9. The recombinant VLP of any one of statements 1 to 6 or 8, wherein the antigen protein and the TM protein domain are from a Spike (S) protein of a SARS-CoV-2.

10. The recombinant VLP of statement 9, wherein the S protein lacks the furin cleavage site at the the S1/S2 boundary and/or the S2' site.
11. The recombinant VLP of any one of statements 1 to 6, wherein the antigen protein and the TM protein domain are from influenza.
12. The recombinant VLP of any one of statements 1 to 6 or 11, wherein the antigen protein is a haemagglutinin (HA) protein or neuroaminidase (NA) protein from influenza.
13. The recombinant VLP of any one of statements 1 to 6, wherein the antigen protein and the TM protein are from a respiratory syncytial virus (RSV).
14. The recombinant VLP of any one of statements 1 to 6 or 13, wherein the antigen protein is a F protein from RSV.
15. The recombinant VLP of any one of statements 1 to 14, wherein the VLP does not include any viral RNA.
16. The recombinant VLP of any one of statements 1 to 15, wherein the VLP has a diameter of between about 70nm and 160nm.
17. The recombinant VLP of statement 16, wherein the VLP has a diameter of about 80nm.
18. The recombinant VLP of any one of statements 1 to 17, wherein the VLP is formulated in a lipid nanoparticle (LNP).
19. An isolated, recombinant or synthetic nucleotide sequence encoding the VLP of any one of statements 1 to 17.
20. An expression vector comprising the nucleotide sequence of statement 19.
21. An immunogenic composition comprising a VLP of any one of statements 1 to 18.
22. A pharmaceutical composition comprising a VLP of any one of statements 1 to 18 and a pharmaceutically acceptable carrier.
23. The pharmaceutical composition of statement 22 for use as a vaccine.

24. A vaccine comprising the pharmaceutical composition of statement 22.
25. The immunogenic composition of statement 21, the pharmaceutical composition of statement 22 or the vaccine of statement 24, further comprising an adjuvant.
26. The immunogenic composition, pharmaceutical composition or the vaccine of statement 25, wherein the adjuvant is MF59.
27. A method of treating or preventing or delaying progression of a disease, disorder or condition in a subject in need thereof, the method comprising administering the VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 to the subject.
28. Use of the VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 in the manufacture of a medicament for treating or preventing or delaying progression of a disease, disorder or condition in a subject.
29. The VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 for use in the treatment or prevention or delaying progression of a disease, disorder or condition.
30. The method of statement 27, the use of statement 28 or the VLP, composition or vaccine for use of statement 29, wherein the disease is a viral infection such as COVID-19, influenza or RSV.
31. A method of inducing an immune response in a subject, the method comprising administering the VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 to the subject in need thereof.
32. Use of the VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

33. The VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 for use in inducing an immune response in a subject in need thereof.

34. The method of statement 31, the use of statement 32, or the VLP, composition or vaccine for use of statement 33, wherein the immune response is a humoral and/or a cell-mediated immune response.

35. The method, the use or the VLP, composition or vaccine for use of statement 34, wherein the immune response is sufficient to treat, prevent or delay progression of at least one symptom of a viral infection caused by a SARS-CoV-2, influenza or RSV.

36. A method for reducing viral load in a subject with a viral infection comprising administering the VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 to the subject in need thereof.

37. Use of the VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 in the preparation of a medicament for reducing viral load in a subject with a viral infection.

38. The VLP of any one of statements 1 to 18, the immunogenic composition of any one of statements 21, 25 or 26, the pharmaceutical composition of any one of statements 22, 23, 25 or 26, or the vaccine of any one of statements 24 to 26 for use in reducing viral load in a subject with a viral infection.

39. The method of any one of statements 27, 30, 31 or 34 to 36, the use of any one of statements 28, 30, 32, 34, 35 or 37, or the VLP, vaccine or composition for use of any one of statements 29, 30, 33, 34, 35 or 38, wherein the subject is a human of 18 years of age or older.

40. The method, the use or the VLP vaccine or composition for use of statement 39, wherein the VLP, vaccine or composition is administered in a one dose regimen.

41. The method, the use or the VLP, vaccine or composition for use of statement 39, wherein the VLP, vaccine or composition is administered in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3 months apart.
42. A eukaryotic cell for expressing the VLP of any one of statements 1 to 17.
43. The cell of statement 42, wherein the cell is a CHO cell or a HEK-293 cell.
44. A method for producing a VLP comprising:
(a) providing one or more expression vectors comprising polynucleotides for expression of the viral like particle (VLP) of any one of statements 1 to 17,
(b) providing a host cell, and
(c) transfecting said host cell with said one or more expression vectors to produce virus-like particles (VLPs) comprising the one or more antigens,
wherein the polynucleotides are expressed under conditions sufficient for the formation of VLPs.
45. The method according to statement 44, further comprising purifying the VLPs.
46. A kit comprising:
(a) the VLP of any one of statements 1 to 18, the pharmaceutical composition of any one of statements 22, 23 or 25 to 26, the immunogenic composition of any one of statements 21, 25 or 26 or the vaccine of any one of statements 24 to 26;
(b) instructions for use thereof; and optionally
(c) a pharmaceutically acceptable carrier, excipient or diluent.

CLAIMS

1. A recombinant virus-like particle (VLP) comprising a capsid fusion protein and a lipid bilayer, the capsid fusion protein comprising:
 - (a) a capsid protein from a non-enveloped virus;
 - (b) a transmembrane (TM) protein domain; and
 - (c) an antigen protein,wherein the capsid protein is encapsulated within the lipid bilayer.
2. The recombinant VLP of claim 1, wherein the capsid protein from a non-enveloped virus is from an alfalfa mosaic virus (AMV), bacteriophage MS2 or bacteriophage AP205.
3. The recombinant VLP of claim 2, wherein the capsid protein is a dimer.
4. The recombinant VLP of claim 1, wherein the capsid protein is fused to the TM protein domain by a peptide linker.
5. The recombinant VLP of claim 1, wherein the TM protein domain and the antigen protein are from a virus.
6. The recombinant VLP of claim 5, wherein the TM protein domain and the antigen protein are from the same virus.
7. The recombinant VLP of claim 5, wherein the TM protein domain and the antigen protein are from different viruses.
8. The recombinant VLP of claim 1, wherein the antigen protein and the TM protein domain are from a SARS-CoV-2.
9. The recombinant VLP of claim 1, wherein the antigen protein and the TM protein domain are from a Spike (S) protein of a SARS-CoV-2.
10. The recombinant VLP of claim 9, wherein the S protein lacks the furin cleavage site at the S1/S2 boundary and/or the S2' site.
11. The recombinant VLP of claim 1, wherein the antigen protein and the TM protein domain are from influenza.

12. The recombinant VLP of claim 1, wherein the antigen protein is a haemagglutinin (HA) protein or neuroaminidase (NA) protein from influenza.
13. The recombinant VLP of claim 1, wherein the antigen protein and the TM protein are from a respiratory syncytial virus (RSV).
14. The recombinant VLP of claim 1, wherein the antigen protein is a F protein from RSV.
15. The recombinant VLP of claim 1, wherein the VLP does not include any viral RNA.
16. The recombinant VLP of claim 1, wherein the VLP has a diameter of between about 70nm and 160nm.
17. The recombinant VLP of claim 16, wherein the VLP has a diameter of about 80nm.
18. The recombinant VLP of claim 1, wherein the VLP is formulated in a lipid nanoparticle (LNP).
19. An isolated, recombinant or synthetic nucleotide sequence encoding the VLP of claim 1.
20. An expression vector comprising the nucleotide sequence of claim 19.
21. An immunogenic composition comprising a VLP of claim 1.
22. A pharmaceutical composition comprising a VLP of claim 1 and a pharmaceutically acceptable carrier.
23. The pharmaceutical composition of claim 22 for use as a vaccine.
24. A vaccine comprising the pharmaceutical composition of claim 22.
25. The immunogenic composition of claim 21, the pharmaceutical composition of claim 22 or the vaccine of claim 24, further comprising an adjuvant.
26. The immunogenic composition, pharmaceutical composition or the vaccine of claim 25, wherein the adjuvant is MF59.

27. A method of treating or preventing or delaying progression of a disease, disorder or condition in a subject in need thereof, the method comprising administering the VLP of claim 1 to the subject.
28. Use of the VLP of claim 1 in the manufacture of a medicament for treating or preventing or delaying progression of a disease, disorder or condition in a subject.
29. The VLP of claim 1 for use in the treatment or prevention or delaying progression of a disease, disorder or condition.
30. The method of claim 27, the use of claim 28 or the VLP for use of claim 29, wherein the disease is a viral infection such as COVID-19, influenza or RSV.
31. A method of inducing an immune response in a subject, the method comprising administering the VLP of claim 1 to the subject in need thereof.
32. Use of the VLP of claim 1 in the manufacture of a medicament for inducing an immune response in a subject in need thereof.
33. The VLP of claim 1 for use in inducing an immune response in a subject in need thereof.
34. The method of claim 31, the use of claim 32, or the VLP for use of claim 33, wherein the immune response is a humoral and/or a cell-mediated immune response.
35. The method, the use or the VLP, composition or vaccine for use of claim 34, wherein the immune response is sufficient to treat, prevent or delay progression of at least one symptom of a viral infection caused by a SARS-CoV-2, influenza or RSV.
36. A method for reducing viral load in a subject with a viral infection comprising administering the VLP of claim 1 to the subject in need thereof.
37. Use of the VLP of claim 1 in the preparation of a medicament for reducing viral load in a subject with a viral infection.
38. The VLP of claim 1 for use in reducing viral load in a subject with a viral infection.
39. The method of claim 27, the use of claim 28, or the VLP for use of claim 29, wherein the subject is a human of 18 years of age or older.

40. The method, the use or the VLP vaccine or composition for use of claim 39, wherein the VLP, vaccine or composition is administered in a one dose regimen.
41. The method, the use or the VLP, vaccine or composition for use of claim 39, wherein the VLP, vaccine or composition is administered in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3 months apart.
42. A eukaryotic cell for expressing the VLP of claim 1.
43. The cell of claim 42, wherein the cell is a CHO cell or a HEK-293 cell.
44. A method for producing a VLP comprising:
(a) providing one or more expression vectors comprising polynucleotides for expression of the viral like particle (VLP) of claim 1,
(b) providing a host cell, and
(c) transfecting said host cell with said one or more expression vectors to produce virus-like particles (VLPs) comprising the one or more antigens,
wherein the polynucleotides are expressed under conditions sufficient for the formation of VLPs.
45. The method according to claim 44, further comprising purifying the VLPs.
46. A kit comprising:
(a) the VLP of claim 1, the pharmaceutical composition of claim 22, the immunogenic composition of claim 21 or the vaccine of claim 24;
(b) instructions for use thereof; and optionally
(c) a pharmaceutically acceptable carrier, excipient or diluent.