(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 May 2024 (16.05.2024)

WIPO | PCT

(10) International Publication Number
WO 2024/100583 A1

(51) International Patent Classification:
A61K 39/12 (2006.01) *C07K 14/005* (2006.01)
A61K 39/155 (2006.01) *A61K 39/00* (2006.01)
A61K 39/42 (2006.01)

(21) International Application Number:
PCT/IB2023/061303

(22) International Filing Date:
09 November 2023 (09.11.2023)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/383,041 09 November 2022 (09.11.2022) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(54) Title: STABILIZED VACCINES

(57) Abstract: The present disclosure relates to a fusion protein comprising an ectodomain of a viral fusion protein linked to one or more heptad repeat(s) (HR(s)) from a SARS-COV-2 spike (S) protein or a respiratory syncytial virus (RSV) F protein, and the uses thereof. The viral fusion proteins are suitable for use as vaccines.

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STABILIZED VACCINES

RELATED APPLICATIONS

This application claims priority from US Patent Application No. 63/383,041 entitled "Stabilized vaccines" filed on 9 November 2022, the entire contents of which are 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 vaccines against viruses and uses thereof.

BACKGROUND

Viral fusion proteins and RNAs encoding same are vaccine candidates because they are the primary targets of protective neutralizing antibody responses for many medically important viruses, e.g., enveloped viruses. However, the intrinsic metastable nature of fusion proteins is an obstacle for effective subunit vaccine design, as recent evidence has shown that broadly cross-reactive and potently neutralizing antibodies elicited during natural infection react primarily with the pre- and not post-fusion forms. In addition, the pre-fusion form of viral fusion proteins contains epitopes that are not present on the post-fusion form (e.g., Magro et al., 2012. Proc. Natl. Acad. Sci. USA 109(8):3089-3094). Thus, for vaccines, the stabilized pre-fusion form is generally considered more desirable antigenically. However, traditional approaches to recombinant expression of these proteins typically results in premature conformational shift to the structurally more stable post-fusion form.

Consequently, there is a pressing need for new approaches to produce stabilized recombinant fusion proteins that remain substantially in their pre-fusion form to stimulate more efficacious immune responses against enveloped viruses.

SUMMARY

In work leading up to the present invention, the inventors sought to produce viral protein ectodomains that were stabilized in a prefusion conformation that were suitable for use as vaccines. To achieve this goal, the inventors fused the viral protein ectodomain to at least one heptad repeat (HR) from a SARS-COV-2 spike (S) protein or at least one

HR from a respiratory syncytial virus (RSV) fusion (F) protein. For example, the inventors produced a fusion protein comprising a RSV F protein ectodomain fused to two HRs, i.e., HR1 and HR2 of SARS-COV-2 S protein. The inventors also produced a fusion protein comprising a SARS-COV-2 S protein ectodomain fused to one HR (HR1) of SARS-COV-2 S protein, and a fusion protein comprising a RSV F protein ectodomain fused to two RSV HRs (HR1 and HR2). The inventors showed that proteins could be expressed and trimerize to form a structure that could induce an immune response against the viral protein when a component of the virus and that the immune response was protective against infection. These findings provide the basis for reagents and methods useful for immunizing subjects against viral infections, e.g., vaccines.

By using the sequence of SARS-COV-2 HRs or RSV HRs, the inventors used a stabilizing structure to which most of the population had been previously exposed either through vaccination or infection by SARS-COV-2 or RSV. Additionally, neither antibodies against SARS-COV-2 S protein nor RSV F protein are generally used as a diagnostic target meaning that vaccination with a fusion protein of the disclosure is unlikely to induce an immune response that may interfere with a diagnostic test.

In one example, the disclosure provides a fusion protein comprising an ectodomain of a viral fusion protein linked to a HR from a SARS-COV-2 S protein or a HR from RSV F protein.

In one example, the disclosure provides a fusion protein comprising an ectodomain of a viral fusion protein linked to a HR from a RSV F protein.

In one example, the disclosure provides a fusion protein comprising an ectodomain of a viral fusion protein linked to a HR from a SARS-COV-2 S protein.

For example, the ectodomain of the viral fusion protein lacks transmembrane and cytoplasmic domains of the protein.

In one example, the HR is additional to any HR(s) present in the ectodomain.

In one example, the HR is HR1 from SARS-COV-2 S protein.

In one example, the HR is HR1 from RSV F protein.

In one example, the ectodomain is linked to two HRs from a SARS-COV-2 S protein. For example, the HRs are HR1 and HR 2 from the SARS-COV-2 S protein.

An exemplary HR1 from SARS-COV-2 comprises a sequence set forth in SEQ ID NO: 1 or 16.

An exemplary HR2 from SARS-COV-2 comprises a sequence set forth in SEQ ID NO: 2 or 16.

In one example, the ectodomain is linked to two HRs from a RSV F protein.

In one example, the HR1 and HR2 are from the RSV F protein.

An exemplary HR1 from RSV comprises a sequence set forth in SEQ ID NO: 14.

An exemplary HR2 from RSV comprises a sequence set forth in SEQ ID NO: 15.

In one exemplary form of the disclosure, the ectodomain is a respiratory syncytial virus F protein ectodomain.

In on example, the ectodomain comprises one or more mutations to stabilize the ectodomain in a prefusion conformation.

In one example, the mutation(s) introduce one or more cysteine residues that form a disulfide bond that is not present in the native ectodomain and/or a mutation that introduces an amino acid that fills a hydrophobic cavity that is present in the native ectodomain.

In on example, the ectodomain comprises one or more of the following groups of mutations that stabilize the F protein ectodomain in a prefusion conformation:

(i) S155C, S290C, S190F and V207L relative to SEQ ID NO: 3;

(ii) N67I and S215P relative to SEQ ID NO: 3;

(iii) N67I, S215P and E487Q relative to SEQ ID NO: 3;

(iv) D486H, E487Q, F488W and D489H relative to SEQ ID NO: 3.

For example, the RSV F protein ectodomain comprises the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3.

In one example, the RSV F protein ectodomain comprises or consists of a sequence set forth in SEQ ID NO: 18.

In one example, the fusion protein comprises in amino to carboxy order:

(i) RSV F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3, HR1 and HR2; or

(ii) RSV F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3, HR2 and HR1.

For example, HR1 and HR2 (or HR2 and HR1) are from RSV.

In one example, the disclosure provides a fusion protein comprising in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3, a linker; HR1 from RSV; and HR2 from RSV.

In one example, the fusion protein comprises in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3, a linker comprising the sequence GGSGGSGGGGSGGSGG (SEQ ID NO: 13); HR1 from RSV and HR2 from RSV.

In one example, the disclosure comprises in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the sequence set forth in SEQ ID NO: 18, a linker comprising the sequence set forth in any one of SEQ ID NOs: 10-13; a HR1 comprising a sequence set forth in SEQ ID NO: 14, a linker, a HR2 comprising a sequence set forth in SEQ ID NO: 15.

In one example, the disclosure comprises in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the sequence set forth in SEQ ID NO: 18, a linker comprising the sequence set forth in SEQ ID NO: 13; a HR1 comprising a sequence set forth in SEQ ID NO: 14, a linker, a HR2 comprising a sequence set forth in SEQ ID NO: 15.

In one example, the fusion protein comprises in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3, a linker comprising the sequence GGSGGSGGGGSGGSGG (SEQ ID NO: 13); HR1 from SARS-COV-2 and HR2 from SARS-COV-2.

In one example, the disclosure comprises in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the sequence set forth in SEQ ID NO: 18, a linker comprising the sequence set forth in any one of SEQ ID NOs: 10-13; a HR1 comprising a sequence set forth in SEQ ID NO: 16, a linker, a HR2 comprising a sequence set forth in SEQ ID NO: 17.

In one example, the disclosure comprises in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the sequence set forth in SEQ ID NO: 18, a linker comprising the sequence set forth in SEQ ID NO: 13; a HR1 comprising a sequence set forth in SEQ ID NO: 16, a linker, a HR2 comprising a sequence set forth in SEQ ID NO: 17.

In one example, the fusion protein comprises a sequence set forth in any one of SEQ ID Nos: 5–9 or 19–23, optionally including a carboxy terminal hexa HIS tag and/or streptavidin tag.

In one example, the fusion protein comprises a sequence set forth in any one of SEQ ID Nos: 19–23.

In one example, the fusion protein comprises a sequence set forth in any one of SEQ ID Nos: 24–28.

In one example, the fusion protein comprises a sequence set forth in SEQ ID NO: 19.

In one example, the fusion protein comprises a sequence set forth in SEQ ID NO: 20.

In one example, the fusion protein comprises a sequence set forth in SEQ ID NO: 21.

In one example, the fusion protein comprises a sequence set forth in SEQ ID NO: 22.

In one example, the fusion protein comprises a sequence set forth in SEQ ID NO: 23.

In one example, the ectodomain is a SARS-COV-2 S protein ectodomain. In this regard, the ectodomain can comprise the sequence as occurs in any variant of SARS-COV-2 or combination of mutations that occur in such variants.

In one example, the SARS-COV-2 ectodomain comprises one or more of the following:

(i) K986P and V987P relative to SEQ ID NO: 4; and/or

(ii) mutation of the furin cleavage site at positions 682 to 685 of SEQ ID NO: 4.

In one example, the furin cleavage site is mutated from RRAR to QQAA or GSAS.

In one example, the fusion protein comprises the SARS-COV-2 S protein ectodomain and one HR of a SARS-COV-2 S protein. For example, the HR is HR1.

In one example, the fusion protein comprises in amino to carboxy terminal order the SARS-COV-2 S protein ectodomain and HR1 of a SARS-COV-2 S protein.

In one example, the fusion protein comprises a sequence set forth in SEQ ID NO: 9.

In one example, the ectodomain and the HR(s) are linked by a linker.

In one example, the linker comprises glycine and serine. For example, the linker comprises the sequence $(GGGGS)_2$ or $(GGGGS)_3$.

In one example, the linker is selected from SEQ ID NOs: 10-13. For example, the linker comprises the sequence set forth in SEQ ID NO: 13.

In one example, the HR1 and HR2 are linked via a further linker.

In one example, the further linker comprises glycine and serine. For example, the further linker comprises the sequence $(GGGGS)_2$ or $(GGGGS)_3$.

In one example, the further linker is selected from SEQ ID NOs: 10–13. For example, the further linker comprises the sequence set forth in SEQ ID NO: 13.

The present disclosure additionally provides a complex or a trimer comprising three of the fusion proteins of the disclosure. For example, the fusion proteins are associated through the HR(s). In one example, the trimer is a homotrimer or the complex is a homocomplex.

The present disclosure additionally provides a nucleic acid encoding the fusion protein of the disclosure. For example, the nucleic acid is a DNA, such as a plasmid. For example, the nucleic acid is a RNA, such as a mRNA or sa-mRNA vaccine.

The disclosure additionally provides a nanoparticle comprising the nucleic acid described herein.

The present disclosure also provides a composition comprising the fusion protein described herein, the nucleic acid described herein or the nanoparticle described herein.

For example, the composition additionally comprises an adjuvant. For example, the adjuvant comprises an oil-in-water emulsion of a squalene, polyoxyethylene sorbitan monooleate and sorbitan trioleate compounds, e.g., MF59.

In one example, the composition comprises a fusion protein comprising in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3, a linker comprising the sequence set forth in SEQ ID NO: 13; HR1 from RSV and HR2 from RSV and adjuvant that is an oil-in-water emulsion of a squalene, polyoxyethylene sorbitan monooleate and sorbitan trioleate compounds.

The disclosure additionally provides a method of inducing an immune response in a subject, the method comprising administering the fusion protein described herein, the nucleic acid described herein, the nanoparticle described herein, or the composition described herein to the subject. In one example, the immune response is against the virus. For example, the immune response is an antibody response. In one example, the immune response is a protective immune response.

The disclosure also provides a method of immunizing a subject, the method comprising administering the fusion protein of described herein, the nucleic acid of described herein, the nanoparticle of described herein or the composition of described herein to the subject.

The disclosure also provides a described herein method of treating or preventing an infection by a virus, the method comprising administering the fusion protein of described herein, the nucleic acid of described herein, the nanoparticle of described herein or the composition of described herein to the subject.

In one example, a fusion protein, composition, nucleic acid or LNP of the disclosure is administered in combination with an influenza vaccine. In one example, the fusion protein, composition, nucleic acid or LNP and influenza vaccine are in the same composition. In another example, the fusion protein, composition, nucleic acid or LNP and influenza vaccine are in separate compositions. In one example, the influenza vaccine is aQIVc, MF59 adjuvanted quadrivalent vaccine grown in cultured cells.

In one example, a fusion protein comprising a RSV F protein ectodomain as described herein is administered together with an influenza vaccine and/or a vaccine against SARS-COV-2.

In one example, a fusion protein comprising a RSV F protein ectodomain as described herein is administered together with a vaccine against PIV3 and/or hMPV.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graphical representation showing a series of fusion proteins comprising the F protein ectodomain comprising mutations at sites S155C, S290C, S190F and V207L (known as "ds Cav1") fused to HR1 and HR2 domains of SARS-COV-2 S protein.

Figures 2A and 2B is a diagrammatic representation showing a cartoon of a antigen (RSV F protein ectodomain) linked to a trimerization domain comprising a HR1 and HR2 from RSV F protein and various linkers as used in the examples herein.

Figure 3A is a graphical representation showing antigen binding IgG titer induced following immunization with a composition comprising a fusion protein comprising a RSV F protein ectodomain linked to a HR1 and HR2 from RSV F protein with the indicated linker and an adjuvant (MF59). Protein was administered at the indicated amount. Statistical differences are indicated. * $p < 0.05$; ** $p < 0.01$; ***$p < 0.005$.

Figure 3B is a graphical representation showing viral neutralisation titer induced following immunization with a composition comprising a fusion protein comprising a RSV F protein ectodomain linked to a HR1 and HR2 from RSV F protein with the indicated linker and an adjuvant (MF59). Protein was administered at the indicated amount. Statistical differences are indicated. * $p < 0.05$; ** $p < 0.01$.

Figure 4 is a diagrammatic representation showing a plan of a RSV challenge experiment in cotton rats using fusion proteins of the disclosure.

KEY TO SEQUENCE LISTING

SEQ ID NO: 1	SARS-COV-2 S protein HR1
SEQ ID NO: 2	SARS-COV-2 S protein HR2
SEQ ID NO: 3	RSV F protein ectodomain
SEQ ID NO: 4	SARS-COV-2 S protein ectodomain
SEQ ID NO: 5	Construct F-6HB V1
SEQ ID NO: 6	Construct F-6HB V2
SEQ ID NO: 7	Construct F-6HB V3

SEQ ID NO: 8 Construct F-6HB V4
SEQ ID NO: 9 Spike-6HB
SEQ ID NO: 10 Linker 1
SEQ ID NO: 11 Linker 2
SEQ ID NO: 12 Linker 3
SEQ ID NO: 13 Linker 4
SEQ ID NO: 14 HR1 from RSV F protein
SEQ ID NO: 15 HR2 from RSV F protein
SEQ ID NO: 16 HR1 from SARS-COV-2
SEQ ID NO: 17 HR2 from SARS-COV-2
SEQ ID NO: 18 RSV ectodomain comprising DS-CAV1 mutations (S155C, S290C, S190F and V207L)
SEQ ID NO: 19 RSV fusion protein (RSV ectodomain – no linker – HR1 from SARS-COV-2 – linker – HR2 from SARS-COV-2)
SEQ ID NO: 20 RSV fusion protein (RSV ectodomain – linker 1 – HR1 from SARS-COV-2 – linker HR2 from SARS-COV-2)
SEQ ID NO: 21 RSV fusion protein (RSV ectodomain – linker 2 – HR1 from SARS-COV-2 – linker HR2 from SARS-COV-2)
SEQ ID NO: 22 RSV fusion protein (RSV ectodomain – linker 3 – HR1 from SARS-COV-2 – linker - HR2 from SARS-COV-2)
SEQ ID NO: 23 RSV fusion protein (RSV ectodomain – linker 4 – HR1 from SARS-COV-2 – linker - HR2 from SARS-COV-2)
SEQ ID NO: 24 RSV fusion protein (RSV ectodomain – no linker – HR1 from SARS-COV-2 – linker – HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)
SEQ ID NO: 25 RSV fusion protein (RSV ectodomain – linker 1 – HR1 from SARS-COV-2 – linker - HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)
SEQ ID NO: 26 RSV fusion protein (RSV ectodomain – linker 2 – HR1 from SARS-COV-2 – linker - HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)
SEQ ID NO: 27 RSV fusion protein (RSV ectodomain – linker 3 – HR1 from SARS-COV-2 – linker HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)

SEQ ID NO: 28	RSV fusion protein (RSV ectodomain – linker 4 – HR1 from SARS-COV-2 – linker HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)
SEQ ID NO: 29	HR1 (HRA) from RSV
SEQ ID NO: 30	HR2 (HRB) from RSV

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 may 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 may 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 may be developed or used from a particular source albeit not necessarily directly from that source.

Selected Definitions

As used herein, "ectodomain" refers to a viral protein (e.g., viral fusion protein) that contains substantially the extracellular portion of the mature viral protein, with or without the signal peptide but lacks the transmembrane domain and cytoplasmic tail. The RSV F ectodomain polypeptide comprises an endogenous HRA domain and an endogenous HRB domain. The SARS-COV-2 S protein comprises an endogenous HR1 domain and an endogenous HR2 domain. For clarification, terms such as "one HR (HR1)" as used throughout the specification refers to one heptad repeat region, and not necessarily the SARS-COV-2 S protein HR1 specifically. For example, HR1 may refer to HRA of RSV or may simply mean a first heptad region, as denoted by context.

The term "linker" or "flexible linker" as used herein refers to a proteinaceous molecule containing at least one amino acid residue, usually at least two amino acids

residues joined by peptide bond(s), which molecule permits two polypeptides linked thereby to move more freely relative to one another, as compared to their movement without the flexible linker. In certain examples, the flexible linker provides increased rotational freedom for two polypeptides linked thereby than the two linked polypeptides would have in the absence of the flexible linker. Such freedom of relative movement or rotational freedom allows polypeptides joined by the flexible linker to perform their individual functions or elicit their activities with less structural hindrance. A flexible linker may be characterized by the absence of secondary structures such as helices or β-sheets or a maximal secondary structure content of 10%, 20% 30% or 40%. Non-limiting examples of flexible linkers include the amino acid sequences GS, GSG, GGS, GGSGG, $(GGS)_2$, GGSG, GSGS, AS, GGGS, $(GGS)_2GG$, $((GGS)_2GG)_2$, G4S, $(G_4S)_2$, $(G_4S)_3$, $(G_4S)_4$, G_4SG, GSGG and GSGGS. Additional flexible linker sequences are known in the art. In various examples, the flexible linker contains or consists of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 amino acid residues. In some examples, the flexible linker contains or consists of up to 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 amino acid residues. In some examples, the flexible linker contains or consists of between about 1 to about 30 amino acid residues, between about 1 to about 25 amino acid residues, between about 1 to about 20 amino acid residues, between about 1 to about 15 amino acid residues, between about 1 to about 12 amino acid residues, between about 1 to about 10 amino acid residues, between about 1 to about 8 amino acid residues, between about 1 to about 6 amino acid residues, between about 1 to about 5 amino acid residues, between about 1 to about 4 amino acid residues, or between about 1 to about 3 amino acid residues. In some examples, the flexible linker contains or consists of between about 2 to about 30 amino acid residues, between about 2 to about 25 amino acid residues, between about 2 to about 20 amino acid residues, between about 2 to about 15 amino acid residues, between about 2 to about 12 amino acid residues, between about 2 to about 10 amino acid residues, between about 2 to about 8 amino acid residues, between about 2 to about 6 amino acid residues, between about 2 to about 5 amino acid residues, or between about 2 to about 4 amino acid residues. In some of the same and other embodiments, the flexible linker contains or consists of between about 3 to about 30 amino acid residues, between about 3 to about 25 amino acid residues, between about 3 to about 20 amino acid residues, between about 3 to about 15 amino acid residues, between about 3 to about 12 amino acid residues, between about 3 to about 10 amino acid residues, between about 3 to about 8 amino acid residues, between about 3 to about 6 amino acid residues, or between about

3 to about 5 amino acid residues. In certain embodiments, the flexible linker contains or consists of 1, 2, 3, 4, 5, 6, 7, 8, 9,10 or 16 amino acid residues.

As used herein, the terms "furin cleavage site" and "furin-like cleavage site" are used interchangeably herein to refer to a scissile bond together with adjacent or non-adjacent recognition elements, or both, sufficient for detectable proteolysis at the scissile bond by furin under conditions suitable for furin protease activity. Furin cleavage sites are known in the art or can be defined by routine methods. See, e.g., Basak, A. et al., 2001. Biochem. J. 353: 537-545; Bader, O. et al., 2008. BMC Microbiol. 8: 116; Schilling, O. et al., 2008. Nat. Biotechnol. 26:685-694 ; Rawlings, N.D. et al., 2008. Nucleic Acids Res. 36(Database issue) : D320-D325; Rawlings, N.D. et al., 2010. Nucleic Acids Res. 38(Database issue) : D227-D233 (2010); Seider, N.G. et al., 2012. Nat. Rev. Drug Discov. 10.1038/nrd3699; Braun, E. et al., 2019. Clin. Transl. Immunol., 8:el073; Izaguirre. G., 2019. Viruses 11 (2019), 10.3390/vll090837. Reference is also made to Coutard, B. et al., 2020. Antiviral Res. 176: 104742, who identified a furin-like cleavage site in SARS-CoV-2.

As used herein, the term “post-fusion conformation” of a fusion protein of a virus refers to the structure of an enveloped virus fusion protein, which is in a terminal conformation (i.e., formed at the end of the fusion process) and is the most energetically favorable state. In the post-fusion conformation, the fusion peptides or loops of the fusion protein are brought into close proximity with the fusion protein transmembrane domain.

As used herein, the term “pre-fusion conformation” of a fusion protein of a virus refers to the structure of a virus fusion protein, which is in a meta-stable confirmation (i.e., in a semi-stable conformation that is not the most energetically favourable terminal conformation) and upon appropriate triggering is able to undergo conformational rearrangement to the terminal post-fusion conformation. Typically, pre-fusion conformations of viral fusion proteins contain a hydrophobic sequence, referred to as the fusion peptide or fusion loop, that is located internally within the pre-fusion conformation and cannot interact with either the viral or host cell membranes. Upon triggering, this hydrophobic sequence is inserted into the host cell membrane and the fusion protein collapses into the post-fusion hairpin like conformation. The pre-fusion conformation of viral fusion proteins vary according to the class of enveloped fusion protein.

As used herein, the term “conventional mRNA” or “cRNA” or “non-amplifying RNA” refers to a construct that allows expression of heterologous RNA and proteins but the RNA that cannot amplify in host cells.

As used herein, the term “self-replicating RNA” refers to a construct based on an RNA virus that has been engineered to allow expression of heterologous mRNA and proteins. Self-replicating RNA (e.g., in the form of naked RNA) can amplify in host cells leading to expression of the desired gene product in the host cell.

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. To facilitate a clear description of the nucleic acids, particular sequence components are referred to as e.g., a “first nucleotide sequence” and a “second nucleotide sequence”. It is to be understood that the first and second sequences can appear in any desired order or orientation, unless otherwise specified, and that no particular order or orientation is intended by the words “first”, “second” etc.

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.

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.

The term "adjuvant" as used herein refers to a compound that, when used in combination with a specific immunogen (e.g., a modified polypeptide, chimeric polypeptide, polypeptide complex, polynucleotide and nucleic acid construct of the present disclosure) in a composition, will augment the resultant immune response, including intensification or broadening the specificity of either or both antibody and cellular immune responses. In the context of the present disclosure, an adjuvant will preferably enhance the specific immunogenic effect of the active agents of the present disclosure. The term "adjuvant" is typically understood not to comprise agents which confer immunity by themselves. An adjuvant assists the immune system non-specifically to enhance the antigen-specific immune response by e.g., promoting presentation of an antigen to the immune system or induction of an unspecific innate immune response.

Furthermore, an adjuvant may e.g., modulate the antigen-specific immune response by e.g., shifting the dominating Th2-based antigen specific response to a more Th1-based antigen specific response or vice versa. Accordingly, an adjuvant may favourably modulate cytokine expression/secretion, antigen presentation, type of immune response etc.

As used herein, the terms "disease", "disorder" or "condition" refers to a disruption of or interference with normal function, and is not to be limited to any specific condition, and will include diseases or disorders.

As used herein, a subject "at risk" of developing a disease or condition may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease 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 disease or condition, as known in the art and/or described herein.

As used herein, the terms "treating", "treat" or "treatment" include administering a protein, a RNA, or composition described herein to thereby reduce or eliminate at least one symptom of a specified disease or condition.

As used herein, the term "preventing", "prevent" or "prevention" includes providing prophylaxis with respect to occurrence or recurrence of a specified disease or condition in an individual. An individual may be predisposed to or at risk of developing the disease but has not yet been diagnosed with the disease.

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 may 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 may 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, e.g., weight or number of RNA. 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" is at least the minimum concentration required to effect a measurable improvement of a particular disease or condition. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the RNA 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 RNA are outweighed by the therapeutically beneficial effects.

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

As used herein, the term "subject" shall be taken to mean any animal including humans, for example a mammal. Exemplary subjects include but are not limited to humans and non-human primates. For example, the subject is a human.

As used herein, the term "lipid nanoparticle" or "LNP" shall be understood to refer to lipid-based particles having at least one dimension on the order of nanometers (e.g., 1–1,000 nm) and which comprises a compound of any formulae described herein. In embodiments, LNPs are formulated in a composition for delivery of a polynucleotide to a desired target such as a cell, tissue, organ, tumour, and the like. For example, the lipid nanoparticle or LNP any lipid composition, including, may be selected from, 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, wherein solid lipid nanoparticles lack lipid bilayers.

RSV F protein

The F protein of RSV directs viral penetration by fusion between the virion envelope and the host cell plasma membrane. It 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 is constantly evolving (Kim et al. (2007) J Med Virol 79: 820-828). Unlike most paramyxoviruses, the F protein in RSV can mediate entry and

syncytium formation independent of the other viral proteins (UN is usually necessary in addition to F in other paramyxoviruses).

The hRSVF mRNA is translated into a 574 amino acid precursor protein designated F0, which contains a signal peptide sequence at the N-terminus that is removed by a signal peptidase in the endoplasmic reticulum. F0 is cleaved at two sites (a.a. 109/110 and 136/137) by cellular proteases (in particular furin) in the trans-Golgi, removing a short glycosylated intervening sequence and generating two subunits designated Fi (~50 kDa; C-terminus; residues 137-574) and F2 (~20 kDa; N- terminus; residues 1-109). Fi contains a hydrophobic fusion peptide at its N-terminus and also two hydrophobic heptad-repeat regions (HRA and HRB, referred to herein as HR1 and HR2, respectively). HRA/HR1 is near the fusion peptide and HRB/HR2 is near to the transmembrane domain. The Fi-F2 heterodimers are assembled as homotrimers in the virion.

The disclosure may use any desired RSV F ectodomain amino acid sequence, such as the amino acid sequence of SEQ ID NO: 3 or a sequence having identity to SEQ ID NO: 3. Typically it will have at least 75% identity to SEQ ID NO: 3 e.g., at least 80%, at least 85%, at least 90%, at least 95%, at least 97%, at least 98%, at least 99%,identity to SEQ ID NO: 1 or 2. The sequence may be found naturally in RSV.

An amino acid sequence within a fusion protein of the disclosure may be found naturally within RSV F ectodomain protein (e.g., a soluble RSV F protein lacking transmembrane and cytoplasmic domains), and/or it may have one or more (e.g. , 1 , 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1 , 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 , 22, 23, 24, 25, 26, 27, 28, 29, 30) single amino acid mutations (insertions, deletions or substitutions) relative to a natural RSV sequence. For instance, it is known to mutate F proteins to eliminate their furin cleavage sequences, thereby preventing intracellular processing.

RSV F polypeptides or proteins may contain one or more mutations that prevent cleavage at one or both of the furin cleavage sites. RSV F ectodomain polypeptides that contain such mutations are not cleaved *in vivo* by cells that produce the polypeptides and are produced as monomers. Examples of suitable furin cleavage mutations to disrupt furin cleavage include replacement of amino acid residues 106–109 of SEQ ID NO: 3 with RARK, RARQ, QAQN, or IEGR. Alternatively, or in addition, amino acid residues 133–136 of SEQ ID NO: 3 can be replaced with RKKK, ΔΔΔR, QNQN, QQQR or IEGR. (Δ indicates that the amino acid residue has been deleted.)

In one example, a RSV F protein ectodomain comprises one or more mutations, e.g., amino acid substitutions to stabilize the ectodomain in its prefusion conformation.

In some examples, the complex is characterized by a rounded (pre-fusion) shape when viewed in negatively stained electron micrographs.

In some examples, the complex is characterised by the ability to bind the D25 antibody which has been characterized as binding to the prefusion structure of RSV-F protein (McClellan *et al., Science. 340(6136)*: 1113–1117 2013).

In another example, the complex comprises prefusion epitopes that are not present on post fusion forms of RSV F protein.

In one example, cysteine residues may be inserted into or substituted into the HRB/HR2 region to form disulfide bonds and stabilize the RSV F ectodomain.

In some examples, the RSV F ectodomain polypeptide includes an S155C mutation and a S290C mutation.

In some examples, the RSV F ectodomain polypeptide includes a mutation at amino acid 190 or amino acid 207. For example, the RSV F ectodomain includes a S190F mutation and/or a V207F mutation.

In some examples, the RSV F ectodomain polypeptide comprises a S155C mutation, a S290C mutation, an S 190F mutation, and a V207F mutation.

In some examples, the RSV F ectodomain polypeptide further includes an internal deletion of all or a portion of the p27 sequence, optionally with a corresponding deletion of one or more furin sites. For example, the RSV F ectodomain includes an internal deletion of about amino acid 103 to about amino acid 136, or about amino acid 103 to 161.

In certain examples, the RSV F ectodomain polypeptide comprises the RSV F sequence of the DS-CAV1 (McClellan *et al., Science. 340(6136)*: 1113–1117 2013). For example, the RSV F protein ectodomain comprises or consists of the sequence set forth in SEQ ID NO: 18.

In some examples, the RSV F complex may be further stabilized in the prefusion form using interchain disulfides including those disclosed in WO2012/158613, using peptides conjugated to oligomerizing agents including but not limited to virus-like particles (VLP's), albumin or RSV G, or using other mutations which further stabilize the monomer so that it retains its prefusion conformation upon formulation and immunization.

In some examples, the RSV F ectodomain may be further stabilized in the prefusion form using disulfide bonds or cavity filling mutations such as disclosed in RSV F McLellan, *et al., Science, 342(6158)*:592-8(2013).

In one example, a RSV F polypeptide, such as an ectodomain polypeptide, may include amino acid changes, relative to SEQ ID NO: 3, of P102A, I379V, M447V, or a combination thereof, e.g., all of P102A, I379V, and M447V.

SARS-COV-2 S protein

In another example, ectodomain is from a SARS-CoV-2 S protein. As discussed above, the present disclosure contemplates the ectodomain of a S protein from any SARS-COV-2 variant. Thus, in one example, the S protein is a mutant S protein.

Suitable mutant S proteins include any occurring in a variant of SARS-COV-2.

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, P479L, P479S, N481D, N481H, V483F, V483A, E484D, E484K, E484K, E484O, 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, E484K, E484O, 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 one example, a mutant S protein comprises a mutation selected from the group consisting of A67V, ΔHV69-70, T95I, G142D, ΔVYY143-145, ΔN211, L212I, R214_D215insEPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F.

In one example, a mutant S protein comprises the following mutations A67V, ΔHV69-70, T95I, G142D, ΔVYY143-145, ΔN211, L212I, R214_D215insEPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F.

In one example, a mutant S protein comprises at least D614G.

In one example, a mutant S protein comprises at least substitutions of two proline residues between residues corresponding to amino acids 986 and 987 of SEQ ID NO: 4.

In one example, the mutant S protein: (i) lacks a furin cleavage site at the S1/S2 boundary and comprises RRAR to QQAA mutations at residues corresponding to nucleotides 682–685 of SEQ ID NO: 4; and/or (ii) lacks a furin cleavage site at the S2' site; and/or (iii) comprises D to G mutation at residue corresponding to nucleotide 614 of SEQ ID NO: 4; and/or (iv) comprises substitutions of two proline residues between residues corresponding to amino acids 986 and 987 of SEQ ID NO: 4.

In one example, the S protein lacks a furin cleavage site at the S1/S2 boundary and comprises RRAR to QQAA mutations at residues corresponding to nucleotides 682-685 of SEQ ID NO: 4.

In one example, the S protein lacks a furin cleavage site at the S2' site.

In one example, the S protein comprises D to G mutation at residue corresponding to nucleotide 614 of SEQ ID NO: 37.

In one example, the S protein comprises insertion of two proline residues between residues corresponding to nucleotides 986 and 987 of SEQ ID NO: 4.

Exemplary mutant S proteins are known in the art and described, for example, in WO2022/008438; WO2022/008438; WO2021/213924; WO2022/155530; WO2022/155524; WO2023/089071; WO2023/094713; WO2023/147091 and/or WO2023147092.

Heptad repeats

As described herein, exemplary fusion proteins of the disclosure comprise a complementary first heptad repeat (HR1) and second heptad repeat (HR2) that associate with each other under conditions suitable for their association (e.g., in aqueous solution) to form an anti-parallel, two-helix bundle.

In some examples of a F protein fusion protein as described herein, the HR1 and HR2 suitably lack complementarity to the HRs of the F protein ectodomain, so that they preferentially form an anti-parallel, two-helix bundle with each other, rather than with structural elements of the ectodomain.

In some examples of a F protein fusion protein as described herein, the HR1 and HR2 are from a F protein ectodomain and form an anti-parallel, two-helix bundle with each other, rather than with structural elements of the ectodomain.

In the case of SARS-COV-2 S protein, a HR1 can be included in the fusion protein that forms an anti-parallel, two-helix bundle with the HR2 in the ectodomain.

In some examples, each of the HR1 and HR2 regions is independently characterized by a n-times repeated 7-residue pattern of amino acid types, represented as $(a\text{-}b\text{-}c\text{-}d\text{-}e\text{-}f\text{-}g\text{-})_n$ or $(d\text{-}e\text{-}f\text{-}g\text{-}a\text{-}b\text{-}c\text{-})_n$, wherein the pattern elements 'a' to 'g' denote conventional heptad positions at which the amino acid types are located and n is a number equal to or greater than 2, and at least 50% (or at least 51% to at least 99% and all integer percentages in between) of the conventional heptad positions 'a' and 'd' are occupied by hydrophobic amino acid types and at least 50% (or at least 51% to at least 99% and all integer percentages in between) of the conventional heptad positions 'b', 'c', 'e', 'f' and 'g' are occupied by hydrophilic amino acid types, the resulting distribution between

hydrophobic and hydrophilic amino acid types enabling the identification of the heptad repeat regions.

A HR1/HRA region that is present in a F polypeptide is the same or substantially the same as the HRA region in the amino acid sequence of the F0 form of the naturally occurring F protein from which the cleaved Fl and F2 peptides are derived. In the case of RSV F proteins, such as an RSV F ectodomain polypeptide or recombinant RSV F ectodomain polypeptide, the endogenous HR1/HRA region is from about amino acid 158 to about amino acid 196 of the amino acid sequence set forth in SEQ ID NO: 3. In some examples, the endogenous HRA is a full length HRA. However, deletions and truncations of the HRA are included within the scope of the disclsoure as long as such deletions and truncations do not substantially alter the ability of RSV F to fold into an acceptable conformation (either pre-fusion or post- fusion) for its intended purpose. A HRA can include one or more mutations such as those discussed herein.

A HRB region that is present in a F polypeptide is the same or substantially the same as the HRB region in the amino acid sequence of the F0 form of the naturally occurring F protein. In the case of RSV F proteins, such as an RSV F ectodomain polypeptide or recombinant RSV F ectodomain polypeptide, the endogenous HRB region is from about amino acid 488 to about amino acid 513 relative to SEQ ID NO: 3. In some examples, the HRB is a full length HRB. However, deletions and truncations of the HRB region are included within the scope of the disclosure as long as such deletions and truncations do not substantially alter the ability of RSV F to fold into an acceptable conformation (either pre-fusion or post- fusion) for its intended purpose. A HRB can include one or more mutations such as those discussed herein.

In specific examples, HR1 and HR2 are from SARS-COV-2. For example, the HR1 comprises a sequence set forth in SEQ ID NO: 1 or 16. For example, the HR2 comprises a sequence set forth in SEQ ID NO: 2 or 17.

For example, the HR1 comprises a sequence set forth in SEQ ID NO: 1. For example, the HR2 comprises a sequence set forth in SEQ ID NO: 2. For example, in a construct comprising a HR1 and HR2 from SARS-COV-2 the HR1 comprises a sequence set forth in SEQ ID NO: 1 and the HR2 comprises a sequence set forth in SEQ ID NO: 2.

For example, the HR1 comprises a sequence set forth in SEQ ID NO: 16. For example, the HR2 comprises a sequence set forth in SEQ ID NO: 17. For example, in a construct comprising a HR1 and HR2 from SARS-COV-2 the HR1 comprises a sequence set forth in SEQ ID NO: 16 and the HR2 comprises a sequence set forth in SEQ ID NO: 17.

In specific examples, the HR1 and HR2 are from RSV. For example, the HR1 comprises a sequence set forth in SEQ ID NO: 14 or 29. For example, the HR2 comprises a sequence set forth in SEQ ID NO: 15 or 30.

For example, the HR1 comprises a sequence set forth in SEQ ID NO: 14. For example, the HR2 comprises a sequence set forth in SEQ ID NO: 15. For example, in a construct comprising a HR1 and HR2 from RSV the HR1 comprises a sequence set forth in SEQ ID NO: 14 and the HR2 comprises a sequence set forth in SEQ ID NO: 15.

For example, the HR1 comprises a sequence set forth in SEQ ID NO: 29. For example, the HR2 comprises a sequence set forth in SEQ ID NO: 30. For example, in a construct comprising a HR1 and HR2 from RSV the HR1 comprises a sequence set forth in SEQ ID NO: 29 and the HR2 comprises a sequence set forth in SEQ ID NO: 30.

In one example, the fusion comprises only one heptad repeat (HR1). For example, the HR1 comprises a sequence set forth in SEQ ID NO: 1 or SEQ ID NO: 14.

Association of the complementary heptad repeats of the structure-stabilizing moiety to one another under conditions suitable for their association (e.g., in aqueous solution) results in formation of an anti-parallel, two-helix bundle that inhibits rearrangement of the modified polypeptide to a post-fusion conformation. This two-helix bundle of the structure-stabilizing moiety can trimerize to form a highly stable six-helix bundle, thus permitting self-assembly of the fusion protein to form a complex. The complex so assembled can mimic the pre-fusion conformation of a wild-type fusion protein e.g., F protein or SARS-CoV-2 S protein complex and comprises three fusion proteins, characterized by a six-helix bundle formed by the coiled coil structures of the respective structure-stabilizing moieties of the chimeric polypeptides.

As discussed above, HR1 and HR2 regions are capable of coming together to form an oligomer, typically a hexamer composed of three HR1 regions and three HR2 regions, which is thermodynamically stable and typifies the post-fusion conformation of class I viral fusion proteins. HR1 and HR2 regions with a strong propensity to oligomerize are referred to herein as "complementary" heptad repeat regions.

Linkers

The fusion protein of the present disclosure suitably comprises a linker that spaces the heptad repeat regions (also referred to herein as HR1 and HR2). The linker generally includes any amino acid residue that cannot be unambiguously assigned to a heptad repeat sequence. Linkers are frequently used in the field of protein engineering to interconnect different functional units. They are generally conformationally flexible in solution, and are suitably and predominantly composed of polar amino acid residue types.

Typical (frequently used) amino acids in flexible linkers are serine and glycine. Less preferably, flexible linkers may also include alanine, threonine and proline. Thus, an intervening linker of the structure-stabilizing moiety is preferably flexible in conformation to ensure relaxed (unhindered) association of HR1 and HR2 as two-helix bundle that suitably adopts an a-helical coiled coil structure. Suitable linkers for use in the polypeptides envisaged herein will be clear to the skilled person, and may generally be any linker used in the art to link amino acid sequences, as long as the linkers are structurally flexible, in the sense that they permit, and suitably do not impair, assembly of the characteristic two-helix bundle structure of the structure-stabilizing moiety.

The intervening linker is suitably an amino acid sequence generally consisting of at least 1 amino acid residue and usually consisting of at least 2 amino acid residues, with a non-critical upper limit chosen for reasons of convenience being about 100 amino acid residues. In particular embodiments, the linker consists of about 1 to about 50 amino acid residues, or about 50 to about 100 amino acid residues, usually about 1 to about 40 amino acid residues, typically about 1 to about 30 amino acid residues. In non-limiting examples, the linker has about the same number of amino acids as the number of amino acids connecting complementary HR1 and HR2 of SARS-COV-2. In non-limiting examples, the linker has about the same number of amino acids as the number of amino acids connecting complementary HR1 and HR2 of RSV.

In particular, non-limiting examples, at least 50% of the amino acid residues of a linker sequence are selected from the group proline, glycine, and serine. In further non-limiting examples, at least 60%, such as at least 70%, such as for example 80% and more particularly 90% of the amino acid residues of a linker sequence are selected from the group proline, glycine, and serine. In other particular examples, the linker sequences essentially consist of polar amino acid residues; in such particular embodiments, at least 50%, such as at least 60%, such as for example 70% or 80% and more particularly 90% or up to 100% of the amino acid residues of a linker sequence are selected from the group consisting of glycine, serine, threonine, alanine, proline, histidine, asparagine, aspartic acid, glutamine, glutamic acid, lysine and arginine. In specific examples, linker sequences may include $[GGSG]_nGG$, $[GGGGS]_n$, $[GGGGG]_n$, $[GGGKGGGG]_n$, $[GGGNGGGG]_n$, $[GGGCGGGG]_n$, wherein n is an integer from 1 to 10, suitably 1 to 5, more suitably 1 to 3. In one example, the linker is selected from SEQ ID NOs: 10-13. For example, the linker comprises the sequence set forth in SEQ ID NO: 13.

Purification tags

In one example, a fusion protein of the disclosure comprises a purification tag. Purification tags typically comprise a stretch of amino acids that enables recovery of the chimeric polypeptide through affinity binding. Numerous purification tags are known in the art, illustrative examples of which include biotin carboxyl carrier protein-tag (BCCP-tag), Myc-tag (c-myc-tag), Calmodulin-tag, FLAG-tag, HA-tag, His-tag (Hexahistidine-tag, His6, 6H), Maltose binding protein-tag (MBP-tag), Nus-tag, Chitin-binding protein-tag (CBP-tag) Glutathione-S-transferase-tag (GST-tag), Green fluorescent protein-tag (GFP-tag), Polyglutamate-tag, Amyloid beta-tag, Thioredoxin-tag, S-tag, Softag 1, Softag 3, Strep-tag.

Complexes

The disclosure additionally provides complexes of a fusion protein described herein. The complexes contain an oligomer of a fusion protein described herein. The term "oligomer" refers to a molecule that consists of more than one but a limited number of monomer units in contrast to a polymer that, at least in principle, consists of an unlimited number of monomers. Oligomers include, but are not limited to, dimers, trimers, tetramers, pentamers, hexamers, heptamers, octamers, nonamers, decamers and the like. An oligomer can be a macromolecular complex formed by non-covalent bonding of macromolecules like proteins. In this sense, a homo-oligomer would be formed by identical molecules and by contrast, a hetero-oligomer would be made of at least two different molecules. In specific examples, an oligomer of the disclosure is a trimeric polypeptide complex consisting of three polypeptide subunits. In these examples, the trimeric polypeptide may be a "homotrimeric polypeptide complex" consisting of three identical polypeptide subunits, or a "heterotrimeric polypeptide complex" consisting of three polypeptide subunits in which at least one subunit polypeptide is non-identical. A "polypeptide subunit" is a fusion protein of the disclosure.

The fusion proteins of the present disclosure can self-assemble under suitable conditions to form complexes. Accordingly, the present disclosure further encompasses a method of producing a complex, wherein the method comprises: combining fusion ptoteins of the present disclosure under conditions (e.g., in aqueous solution) suitable for the formation of a complex, whereby a complex is produced that comprises three fusion proteins. The fusion proteins that are combined may be identical or non-identical to thereby form homotrimers and heterotrimers, respectively.

Generally, the fusion proteins self-assemble in a buffered aqueous solution (e.g., pH about 5 to about 9). If required, mild denaturing conditions can be used, such as, by

including urea, small amounts of organic solvents or heat to mildly denature the fusion proteins in order to facilitate refolding and self-assembly.

Any suitable preparation of fusion proteins can be used in the method. For example, conditioned cell culture media that contains the desired fusion protein can be used in the method. However, it is desirable to use purified fusion protein in the method. Methods to detect oligomer formation are well in the art and include, e.g., Western blot using non-boiled, non-reduced samples; size exclusion chromatography including size exclusion chromatography in conjunction with antibody binding. Although such methods do not permit direct observation of the six-helix bundle, the formation of an oligomer without significant formation higher order structures as determined by Western blot, can be understood to be an indication of the formation of a six helix bundle. In size exclusion chromatography, the formation of a complex of the appropriate mobility for an oligomer of interest peak at the appropriate position without a significant shoulder without significant flow through indicative of higher order structures. Appropriate controls to determine the mobility of trimers (e.g., DS-CAV1 with a foldon trimerization domain as provided in McClellan et al., 2013) and monomers (e.g., delta p23 furdel) can be readily identified by those of skill in the art. In an example, at least 60% of the fusion protein s(by weight) in a sample are present in a trimer. In another example, at least 70% of the fusion proteins (by weight) in a sample are present in a trimer. In another example, at least 75%> of the fusion proteins (by weight) in a sample are present in a trimer. In a further example, at least 80% of the fusion proteins (by weight) in a sample are present in a trimer. In another example, at least 85% of the fusion proteins (by weight) in a sample are present in a trimer. In a further example, at least 90%> of the fusion proteins (by weight) in a sample are present in a trimer. In a still further example, at least 95% of the fusion proteins (by weight) in a sample are present in a trimer. Methods such as Western blot can be used to determine the amount of protein present in monomer and trimer forms. Those of skill in the art understand the use of proper control and standard samples to provide quantitative results, or results from western blots that would permit determination of the relative portion of the protein present in a monomer, trimer, or other form. Similarly, chromatography methods can be performed with appropriate controls to determine at least the relative amounts of protein in monomers, trimer, and other forms.

Compositions and vaccines

The disclosure additionally provides compositions that comprise the fusion proteins disclosed herein. The compositions are suitable for administration to a mammalian subject, such as a human, and may include one or more pharmaceutically

acceptable carrier(s) and/or excipient(s), including adjuvants. A thorough discussion of such components is available in Gennaro (2000) Remington: The Science and Practice of Pharmacy. 20th edition, ISBN: 0683306472. Compositions will generally be in aqueous form. When the composition is an immunogenic composition or vaccine, it will elicit an immune response when administered to a mammal, such as a human. In some examples, in the case of a vaccine, the immune response is a neutralizing immune response or a protective immune response.

The compositions may include a single active ingredient e.g., fusion protein, or several active ingredients. For example, the composition the composition comprises a RSV-F fusion protein and a SARS-COV-2 S fusion protein. In another example, the composition comprises SARS-COV-2 S fusion proteins wherein the S protein ectodomains are from different SARS-COV-2 variants. For example, the compositions can contain one or more immunogens from other pathogens, e.g., influenza.

The composition may include preservatives such as thiomersal or 2-phenoxyethanol.

To control tonicity, a composition can comprise a physiological salt, such as a sodium salt. Sodium chloride (NaCl) is exemplary, which may be present at between 1 and 20 mg/ml. Other salts that may be present include potassium chloride, potassium dihydrogen phosphate, disodium phosphate dehydrate, magnesium chloride, calcium chloride, and the like.

Compositions will generally have an osmolality of between 200 mOsm/kg and 400 mOsm/kg, such as between 240-360 mOsm/kg, for example, within the range of 290-310mOsm/kg.

Compositions may include one or more buffers. Typical buffers include: a phosphate buffer; a Tris buffer; a borate buffer; a succinate buffer; a histidine buffer (particularly with an aluminium hydroxide adjuvant); or a citrate buffer. Buffers will typically be included in the 5- 20mM range.

The pH of a composition will generally be between 5.0 and 8.1, and more typically between 6.0 and 8.0, e.g, between 6.5 and 7.5, or between 7.0 and 7.8.

In one example, the composition is sterile. The composition is preferably non-pyrogenic, e.g., containing <1 EU (endotoxin unit, a standard measure) per dose, and preferably <0.1 EU per dose. The composition is preferably gluten free. Human vaccines are typically administered in a dosage volume of about 0.5ml, although a half dose (i.e., about 0.25ml) may be administered to children.

In one example, the composition comprises an adjuvant. According to the disclosure adjuvants can be, but are not limited to, organic, inorganic, oil-based adjuvants or virosomes.

Inorganic adjuvants include, but are not limited to mineral adjuvants, for example aluminium or calcium salts, such as aluminium phosphate, aluminium hydroxide (also referred to as $Al(OH)_3$ herein), potassium aluminium sulphate (also referred to as alum) and calcium phosphate. Such adjuvants may be used with or without other adjuvants. Organic adjuvants include, but are not limited, to squalene.

Further examples of adjuvants according to the disclosure include, but are not limited to, MPL (Monophosphoryl Lipid A), AS03 (developed by GSK, Prepandrix), AS04 (developed by GSK; combination of MPL and aluminum hydroxide; Fendrix; Cervarix), QS21 (Saponin purified plant extract from the Soap bark tree (Quillaia saponaria) containing triterpene glucoside), AS01 (developed by GSK; liposomes; QS21 and MPL), AS02 (developed by GSK; QS21 and MPL), LT (heat labile enterotoxin from E.coli), CpG (oligonucleotides containing unmethylated CpG sequences), and MF59 (from Novartis). MF59 is a sub-micron oil-in-water emulsion of a squalene, polyoxyethylene sorbitan monooleate and sorbitan trioleate compounds.

Adjuvants suitable for the disclosure are for example mineral adjuvants or adjuvants containing squalene, e.g. emulsion of squalene, e.g. MF59. In one example, the composition of the disclosure comprises a fusion protein of the disclosure and either (i) MF59 or (ii) an aluminium salt (such as aluminium hydroxide). In one example, the composition of the disclosure comprises a fusion protein of the disclosure and MF59.

The choice of adjuvant depends on the efficiency of adjuvant in promoting the immune response, the stability of the composition containing the adjuvant, e.g. the vaccine containing the adjuvant, the route of administration, the dosing regimen, the species to be vaccinated.

Two or more adjuvants can be combined. For example, aluminium salts can be combined with MPL, QS21, and/or MF59.

Nucleic acids

The disclosure additionally provides nucleic acids encoding a fusion protein of the disclosure.

In some examples, the nucleic acid is a mRNA or sa-mRNA encoding the fusion protein Nucleic acids useful in the present disclosure may include a first region of linked nucleosides encoding a polypeptide of interest (e.g., a coding region), a first flanking region located at the 5'-terminus of the first region (e.g., a 5'-UTR), a second flanking

region located at the 3'-terminus of the first region (e.g., a 3'-UTR), at least one 5'-cap region, and a 3'-stabilizing region. In some examples, a nucleic acid further includes a poly-A region or a Kozak sequence (e.g., in the 5'-UTR). In some cases, nucleic acids may contain one or more intronic sequences capable of being excised from the nucleic acid. In some examples, a nucleic acid (e.g., an mRNA) may include a 5' cap structure, a chain terminating nucleotide, a stem loop, a poly A sequence, and/or a polyadenylation signal. Any one of the regions of a nucleic acid may include one or more alternative components (e.g., an alternative nucleoside). For example, the 3'-stabilizing region may contain an alternative nucleoside such as an L-nucleoside, an inverted thymidine, or a 2'-O-methyl nucleoside and/or the coding region, 5'-UTR, 3'-UTR, or cap region may include an alternative nucleoside such as a 5-substituted uridine (e.g., 5-methoxy uridine), a 1-substituted pseudouridine (e.g., 1-methyl-pseudouridine or 1-ethyl-pseudouridine), and/or a 5-substituted cytidine (e.g., 5-methyl-cytidine).

Nucleic acids suitable for use with the present LNPs may include one or more naturally occurring components, including any of the canonical nucleotides A (adenosine), G (guanosine), C (cytosine), U (uridine), or T (thymidine). In one embodiment, all or substantially all of the nucleotides comprising (a) the 5'-UTR, (b) the open reading frame (ORF), (c) the 3'-UTR, (d) the poly A tail, and any combination of (a, b, c, or d above) comprise naturally occurring canonical nucleotides A (adenosine), G (guanosine), C (cytosine), U (uridine), or T (thymidine).

In some examples, nucleic acids may include one or more alternative components, as described herein, which impart useful properties including increased stability and/or the lack of a substantial induction of the innate immune response of a cell into which the nucleic acid is introduced. For example, an alternative nucleic acid exhibits reduced degradation in a cell into which the nucleic acid is introduced, relative to a corresponding unaltered nucleic acid. These alternative species may enhance the efficiency of protein production, intracellular retention of the nucleic acids, and/or viability of contacted cells, as well as possess reduced immunogenicity.

Nucleic acids may be naturally or non-naturally occurring. Nucleic acids may include one or more modified (e.g., altered or alternative) nucleobases, nucleosides, nucleotides, or combinations thereof. The nucleic acids may include any useful modification or alteration, such as to the nucleobase, the sugar, or the internucleoside linkage (e.g., to a linking phosphate / to a phosphodiester linkage / to the phosphodiester backbone). In some embodiments, one or more alterations are present in each of the nucleobase, the sugar, and the internucleoside linkage.

Nucleic acids may or may not be uniformly altered along the entire length of the molecule. For example, one or more or all types of nucleotide (e.g., purine or pyrimidine, or any one or more or all of A, G, U, C) may or may not be uniformly altered in a nucleic acid, or in a given predetermined sequence region thereof.

Different sugar alterations and/or internucleoside linkages (e.g., backbone structures) may exist at various positions in a nucleic acid. One of ordinary skill in the art will appreciate that the nucleotide analogs or other alteration(s) may be located at any position(s) of a nucleic acid such that the function of the nucleic acid is not substantially decreased. An alteration may also be a 5'- or 3 '-terminal alteration. In some embodiments, the nucleic acid includes an alteration at the 3'-terminus.

Lipid Nanoparticles

The present disclosure provides for an LNP for delivery of a nucleic acid, such as a RNA or sa-mRNA encoding a fusion protein of the disclosure.

In examples, the LNPs have a mean diameter of from about 30 nm to about 160 nm, from about 40 nm to about 160 nm, from about 50 nm to about 160 nm, from about 60 nm to about 160 nm, from about 70 nm to about 160 nm, from about 50 nm to about 140 nm, from about 60 nm to about 130 nm, from about 70 nm to about 120 nm, from about 80 nm to about 120 nm, from about 90 nm to about 120 nm, from about 70 to about 110 nm, from about 80 nm to about 110 nm, or about 40 nm, 45 nm, 50 nm, 55 nm, 60 nm, 65 nm, 70 nm, 75 nm, 80 nm, 85 nm, 90 nm, 95 nm, 100 nm, 105 nm, 110 nm, 115 nm, 120 nm, 125 nm, 130 nm, 135 nm, 140 nm, 145 nm, 150 nm, 155 nm or 160 nm. The diameter of the LNP may be measured by dynamic light scattering (DLS), transmission electron microscopy (TEM), scanning electron microscopy (SEM), or other methods such as are known in the art.

In some examples, the LNPs may be relatively homogenous. A polydispersity index may be used to indicate the homogeneity of the LNPs. A small, for example less than 0.3 or less than 0.2, polydispersity index generally indicates a narrow particle size distribution. A composition of the LNPs described herein may have a polydispersity index from about 0 to about 0.25, such as 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.11, 0.12, 0.13, 0.14, 0.15, 0.16, 0.17, 0.18, 0.19, 0.20, 0.21, 0.22, 0.23, 0.24, or 0.25. In some embodiments, the polydispersity index of the LNP composition may be from about 0 to about 0.20 or 0.05 to 0.20.

The LNP may comprise a cationic and/or ionizable lipid, a netral lipid, a PEG-lipid and a sterol.

The LNP may comprise a cationic and/or ionizable lipids selected from the non-limiting group consisting of:

- 3-(didodecylamino)-N1,N1,4-tridodecyl-1-piperazineethanamine (KL10),
- N1-[2-(didodecylamino)ethyl]-N1,N4,N4-tridodecyl-1,4-piperazinediethanamine (KL22),
- 14,25-ditridecyl-15,18,21,24-tetraaza-octatriacontane (KL25),
- 1,2-dilinoleyloxy-N,N-dimethylaminopropane (DLin-DMA),
- 2,2-dilinoleyl-4-dimethylaminomethyl-[1,3]-dioxolane (DLin-K-DMA),
- (6Z,9Z,28Z,31Z)-heptatriacont-6,9,28,31-tetraene-19-yl 4-(dimethylamino)butanoate (DLin-MC3-DMA),
- 2,2-dilinoleyl-4-(2-dimethylaminoethyl)-[1,3]-dioxolane (DLin-KC2-DMA),
- 1,2-dioleyloxy-N,N-dimethylaminopropane (DODMA),
- 2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-dien-1-y loxy]propan-1-amine (Octyl-CLinDMA),
- (2R)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-die n-1-yloxy]propan-1-amine (Octyl-CLinDMA (2R)),
- (2S)-2-({8-[(3β)-cholest-5-en-3-yloxy]octyl}oxy)-N,N-dimethyl-3-[(9Z,12Z)-octadeca-9,12-die n-1-yloxy]propan-1-amine (Octyl-CLinDMA (2S)),
- ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)) and
- 8-[(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino]-octanoic acid, 1-octylnonyl ester.

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 embodiment, a PEG lipid includes PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, a PEG-DSPE lipid and combinations thereof.

Suitable neutral or zwitterionic lipids for use in the present disclosure will be apparent to the skilled person and include, in embodiments, 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 (OChemsPC), 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.

Exemplary structural lipids or sterols 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. In embodiments, the structural lipid is cholesterol. In another embodiment, the structural lipid is campesterol.

Methods of treatment and administration

Compositions of the disclosure are suitable for administration to mammals, e.g., humans, and the invention provides a method of inducing an immune response in a mammal, comprising administering a composition (e.g., an immunogenic composition) or a fusion protein or a nucleic acid encoding same of the disclosure to the mammal. In certain examples, the immune response is a neutralizing immune response. The compositions (e.g., an immunogenic composition) can be used to produce a vaccine formulation for immunizing a mammal. The mammal is typically a human.

The disclosure also provides a composition for use as a medicament, e.g., for use in immunizing a patient against a viral infection, e.g., RSV infection or SARS-COV-2 infection, e.g., for use in raising a neutralizing immune response in a patient.

The immune response raised by these methods and uses will generally include an antibody response, preferably a protective antibody response (i.e., a neutralizing response).

Methods for assessing antibody responses after vaccination are known in the art. Compositions of the invention can be administered in a number of suitable ways, such as intramuscular injection (e.g., into the arm or leg), subcutaneous injection, intranasal administration, oral administration, intradermal administration, transcutaneous administration, transdermal administration, and the like. The appropriate route of administration will be dependent upon the age, health and other characteristics of the

mammal. A clinician will be able to determine an appropriate route of administration based on these and other factors.

Immunogenic compositions, and vaccine formulations, may be used to treat children and adults, including pregnant women. Thus, a subject may be less than 1 year old, 1-5 years old, 5-15 years old, 15-55 years old, or at least 55 years old. Preferred subjects for receiving the vaccines are the elderly (e.g., >50 years old, >60 years old, and preferably >65 years) and pregnant women. The vaccines are not suitable solely for these groups, however, and may be used more generally in a population.

Treatment can be by a single dose schedule or a multiple dose schedule. Multiple doses may be used in a primary immunization schedule and/or in a booster immunization schedule. In a multiple dose schedule the various doses may be given by the same or different routes, e.g., a parenteral prime and mucosal boost, a mucosal prime and parenteral boost, etc. Administration of more than one dose (typically two doses) is particularly useful in immunologically naive patients.

Multiple doses will typically be administered at least 1 week apart (e.g., about 2 weeks, about 3 weeks, about 4 weeks, about 6 weeks, about 8 weeks, about 10 weeks, about 12 weeks, about 16 weeks, and the like.).

The present disclosure includes the following non-limiting Examples.

EXAMPLES

Example 1: RSV-F protein ectodomain – SARS-COV-2 HRs fusion proteins

Fusion proteins comprising the DS Cav1 mutant form of RSV F protein ectodomain (comprising S155C, S290C, S190F and V207L mutations) linked to HR1 and HR2 (in either order) of SARS-COV-2 were produced (Figure 1). mRNAs encoding the fusion protein were also produced and encapsulated in LNPs. Protein was administered with MF59 adjuvant. Administration of the fusion protein or the RNA to mice elicited a dose dependent anti-F protein antibody response that was shown to be neutralising.

Example 2: SARS-COV-2 S protein ectodomain – SARS-COV-2 HRs fusion proteins

Fusion proteins comprising a SARS-COV-2 S protein ectodomain fused to a HR1 of SARS-COV-2 S protein or fused to a HR1 and HR2 of SARS-COV-2 S protein were produced (Figure 1). The SARS-COV-2 S protein ectodomain comprised proline substitutions at positions K986P and V987P relative to SEQ ID NO: 4 and a mutation of the furin cleavage site at positions 682 to 685 from RRAR to QQAA or GSAS. A fusion

protein comprising only the HR1 comprises the sequence set forth in SEQ ID NO: 9. mRNAs encoding the fusion proteins were also produced.

The inventors found that the fusion protein comprising only the HR1 was produced at a higher level and was more stable than the fusion protein comprising the HR1 and HR2. This was somewhat surprising since the HR1 domain in the fusion protein would likely interact with the HR2 in the ectodomain.

The inventors additionally found that administration of the fusion protein or sa-mRNA encoding same (optionally with MF59 adjuvant) induced an antibody response against various SARS-COV-2 variants. Moreover, the inventors found that the fusion protein comprising only the HR1 was more immunogenic than the fusion protein comprising the HR1 and HR2.

Example 3: Linkers

Trimeric recombinant protein antigens comprising RSV-F protein ectodomain linked to SARS-COV-2 HR1 and HR2 were generated with varied linker length between the antigenic region for the protein and the trimerization domain (Figure 2). The linkers ranged from 0 to 16 amino acids in length. Trimeric recombinant protein antigens comprising RSV-F protein ectodomain linked to RSV HR1 and HR2 with the same linker combinations ranging from 0 to 16 amino acids in length can also be generated. Examples of some of the linkers tested are described in Table 1 below.

Table 1

Linker	**Sequence**	**SEQ ID NO:**
No linker	—	—
Linker 1	GGS	10
Linker 2	GGSGGS	11
Linker 3	GGSGGSGG	12
Linker 4	GGSGGSGGGGSGGSGG	13

Example 4: Linker length affects immunogenicity

Trimeric recombinant protein antigens with different linker length (Table 1) were administered to mice (optionally with MF59 adjuvant). Fusion proteins were administered in doses ranging from 1 μg to 0.1 μg. ELISA was performed to confirm IgG binding. Induction of binding IgG was observed to vary depending on linker length (Figure 3 A). Neutralisation titres were also observed to vary depending on linker length (Figure 3 B). 0.1 μg is the preferred dose, advantageously providing good binding and

neutralisation, especially when Linker 4 (GGSGGSGGGGSGGSGG; SEQ ID NO: 13) is used in the fusion protein.

Example 5: Cotton rat RSV challenge study design

Pre-clinical testing will involve a cotton rat study (Figure 4). After acclimation female cotton rats (CR) aged 6 weeks are immunised twice, 3 weeks apart with vaccine or saline control. Four weeks post-boost all CRs will be challenged intranasally with 10^6 plaque forming units (PFU) of RSV A2 virus. Nasal swabs are collected daily, once before the viral challenge and for 5 days following viral challenge for quantification of viral loads by real-time quantitative polymerase chain reaction (RTqPCR). Sera are collected for immunogenicity testing the day prior to immunisation (by vaccine or saline), at day 20 post-immunisation (vaccine or saline) at day 48 post-immunisation (vaccine or saline), and at day 54 post-immunisation (vaccine or saline). On study day 54 (5 days post-challenge) animals are euthanised and their blood collected for serological assays. Lungs and nasal tissues are collected for viral loads by PFU analysis and RTqPCR.

Table of sequences

SEQ	Name	Sequence
1	SARS-COV-2 S protein HR1	AIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVKQLS SNFGAISSVL
2	SARS-COV-2 S protein HR2	DVLGDISGINASVVNIQKEIDRLNEVAKNLNES
3	RSV F protein ectodomain	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPPTNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTSK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEINLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGMDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELL
4	SARS-COV-2 S protein ectodomain	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYY PDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGTNGT KRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQ SLLIVNNATNVVIKVCEFQFCNDPFLGVYYHKNNKSW MESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNL REFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVD LPIGINITRFQTLLALHRSYLTPGDSSSGWTAGAAAYYV GYLQPRTFLLKYNENGTITDAVDCALDPLSETKCTLKS FTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNAT RFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVS PTKLNDLCFTNVYADSFVIRGDEVRQIAPGQTGKIADY NYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLFRK SNLKPFERDISTEIYQAGSTPCNGVEGFNCYFPLQSYGF QPTNGVGYQPYRVVVLSFELLHAPATVCGPKKSTNLV KNKCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADT TDAVRDPQTLEILDITPCSFGGVSVITPGTNTSNQVAVL YQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAG

SEQ	Name	Sequence
		CLIGAEHVNNSYECDIPIGAGICASYQTQTNSPRRARSV ASQSIIAYTMSLGAENSVAYSNNSIAIPTNFTISVTTEILP VSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRA LTGIAVEQDKNTQEVFAQVKQIYKTPPIKDFGGFNFSQI LPDPSKPSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDI AARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTIT SGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQ KLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQNAQA LNTLVKQLSSNFGAISSVLNDILSRLDKVEAEVQIDRLIT GRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLGQ SKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEK NFTTAPAICHDGKAHFPREGVFVSNGTHWFVTQRNFYE PQIITTDNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEE LDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVA KNLNESLIDLQ
5	Construct F-6HB V1	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPPTNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEINLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGMDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGSGGSGGENQKLIANQFNSAIGKIQ DSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAI SSVLGGSGGSGGDVDLGDISGINASVVNIQKEIDRLNEV AKNLNESGGSGGSLVPRGGSAGSGWSHPQFEKGGGSG GGSGGGSWSHPQFEKGSKGG
6	Construct F-6HB V2	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI

SEQ	Name	Sequence
		KQELDKYKNAVTELQLLMQSTPPTNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEINLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGMDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGSGGSGGSAIGKIQDSLSSTASALG KLQDVVNQNAQALNTLVKQLSSNFGAISSVLGGSGGS GGDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESGG SGGSLVPRGGSAGSGWSHPQFEKGGGSGGGSGGGSWS HPQFEKGSKGG
7	Construct F-6HB V3	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGGGSGGGGSGGGGSGVTQNVLYE NQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNHNA QALNTLVKQLSSKFGAISSVLNDIFSRLDGGGGSGGGG SDVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDL QELGKSSGSSG

SEQ	Name	Sequence
8	Construct F-6HB V4	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGGGSGGGGSGGGGSDVDLGDISGI NASVVNIQKEIDRLNEVAKNLNESLIDLQELGKGGGGS GGGGSGVTQNVLYENQKLIANQFNSAIGKIQDSLSSTA SALGKLQDVVNHNAQALNTLVKQLSSKFGAISSVLNDI FSRLDSSGSSG
9	Spike-6HB	MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYY PDKVFRSSVLHSTQDLFLPFFSNVTWFHVISGTNGTKRF DNPVLPFNDGVYFASIEKSNIIRGWIFGTTLDSKTQSLLI VNNATNVVIKVCEFQFCNDPFLDHKNNKSWMESEFRV YSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNI DGYFKIYSKHTPIIVREPEDLPQGFSALEPLVDLPIGINIT RFQTLLALHRSYLTPGDSSSGWTAGAAAYYVGYLQPR TFLLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGI YQTSNFRVQPTESIVRFPNITNLCPFDEVFNATRFASVY AWNRKRISNCVADYSVLYNLAPFFTFKCYGVSPTKLN DLCFTNVYADSFVIRGDEVRQIAPGQTGNIADYNYKLP DDFTGCVIAWNSNKLDSKVSGNYNYLYRLFRKSNLKP FERDISTEIYQAGNKPCNGVAGFNCYFPLRSYSFRPTYG VGHQPYRVVVLSFELLHAPATVCGPKKSTNLVKNKCV NFNFNGLKGTGVLTESNKKFLPFQQFGRDIADTTDAVR DPQTLEILDITPCSFGGVSVITPGTNTSNQVAVLYQGVN CTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAE

SEQ	Name	Sequence
		YVNNSYECDIPIGAGICASYQTQTKSHGSASSVASQSIIA YTMSLGAENSVAYSNNSIAIPTNFTISVTTEILPVSMTKT SVDCTMYICGDSTECSNLLLQYGSFCTQLKRALTGIAV EQDKNTQEVFAQVKQIYKTPPIKYFGGFNFSQILPDPSK PSKRSFIEDLLFNKVTLADAGFIKQYGDCLGDIAARDLI CAQKFKGLTVLPPLLTDEMIAQYTSALLAGTITSGWTF GAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIAN QFNSAIGKIQDSLSSTASALGKLQDVVNHNAQALNTLV KQLSSKFGAISSVLNDIFSRLDPPEAEVQIDRLITGRLQS LQTYVTQQLIRAAEIRASANLAATKMSECVLGQSKRVD FCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNFTTA PAICHDGKAHFPREGVFVSNGTHWFVTQRNFYEPQIITT DNTFVSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKY FKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLN ESLIDLQELGKYEggggsggggsggggsGVTQNVLYENQKLI ANQFNSAIGKIQDSLSSTASALGKLQDVVNHNAQALNT LVKQLSSKFGAIssgssg
10	Linker 1	GGS
11	Linker 2	GGSGGS
12	Linker 3	GGSGGSGG
13	Linker 4	GGSGGSGGGGSGGSGG
14	HR1 (HRA) from RSV	LHLEGEVNKIKSALLSTNKAVVSLGNGVSVLTSKVLDL K
15	HR2 (HRB) from RSV	FDASISQVNEKINQSLAFIRKSDELL
16	HR1 from SARS-COV-2	ENQKLIANQFNSAIGKIQDSLSSTASALGKLQDVVNQN AQALNTLVKQLSSNFGAISSVL
17	HR2 from SARS-COV-2	DVDLGDISGINASVVNIQKEIDRLNEVAKNLNES
18	RSV ectodomain comprising DS-CAV1 mutations (S155C, S290C, S190F and V207L)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI

SEQ	Name	Sequence
		DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELL
19	RSV fusion protein (RSV ectodomain – no linker – HR1 from SARS-COV-2 – linker – HR2 from SARS-COV-2)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLENQKLIANQFNSAIGKIQDSLSSTASA LGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLGGSG GSGGDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES
20	RSV fusion protein (RSV ectodomain – linker 1 – HR1 from SARS-COV-2 – linker HR2 from SARS-COV-2)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN

SEQ	Name	Sequence
		QSLAFIRKSDELLGGSENQKLIANQFNSAIGKIQDSLSST ASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLG GSGGSGGDVDLGDISGINASVVNIQKEIDRLNEVAKNL NES
21	RSV fusion protein (RSV ectodomain – linker 2 – HR1 from SARS-COV-2 – linker HR2 from SARS-COV-2)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGSGGSENQKLIANQFNSAIGKIQDS LSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS VLGGSGGSGGDVDLGDISGINASVVNIQKEIDRLNEVA KNLNES
22	RSV fusion protein (RSV ectodomain – linker 3 – HR1 from SARS-COV-2 – linker - HR2 from SARS-COV-2)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGSGGSGGENQKLIANQFNSAIGKIQ

SEQ	Name	Sequence
		DSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAI SSVLGGSGGSGGDVDLGDISGINASVVNIQKEIDRLNEV AKNLNES
23	RSV fusion protein (RSV ectodomain – linker 4 – HR1 from SARS-COV-2 – linker - HR2 from SARS-COV-2)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGSGGSGGGGSGGSGGENQKLIANQ FNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVK QLSSNFGAISSVLGGSGGSGGDVDLGDISGINASVVNIQ KEIDRLNEVAKNLNES
24	RSV fusion protein (RSV ectodomain – no linker – HR1 from SARS-COV-2 – linker – HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLENQKLIANQFNSAIGKIQDSLSSTASA LGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLGGSG

SEQ	Name	Sequence
		GSGGDVDLGDISGINASVVNIQKEIDRLNEVAKNLNES GGSGGSLVPRGGSAGSGWSHPQFEKGGGSGGGSGGGS WSHPQFEKGSKGGHHHHHH
25	RSV fusion protein (RSV ectodomain – linker 1 – HR1 from SARS-COV-2 – linker - HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGSENQKLIANQFNSAIGKIQDSLSST ASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSVLG GSGGSGGDVDLGDISGINASVVNIQKEIDRLNEVAKNL NESGGSGGSLVPRGGSAGSGWSHPQFEKGGGSGGGSG GGSWSHPQFEKGSKGGHHHHHH
26	RSV fusion protein (RSV ectodomain – linker 2 – HR1 from SARS-COV-2 – linker - HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGSGGSENQKLIANQFNSAIGKIQDS

SEQ	Name	Sequence
		LSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISS VLGGSGGSGGDVDLGDISGINASVVNIQKEIDRLNEVA KNLNESGGSGGSLVPRGGSAGSGWSHPQFEKGGGSGG GSGGGSWSHPQFEKGSKGGHHHHHH
27	RSV fusion protein (RSV ectodomain – linker 3 – HR1 from SARS-COV-2 – linker HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELLGGSGGSGGENQKLIANQFNSAIGKIQ DSLSSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAI SSVLGGSGGSGGDVDLGDISGINASVVNIQKEIDRLNEV AKNLNESGGSGGSLVPRGGSAGSGWSHPQFEKGGGSG GGSGGGSWSHPQFEKGSKGGHHHHHH
28	RSV fusion protein (RSV ectodomain – linker 4 – HR1 from SARS-COV-2 – linker HR2 from SARS-COV-2 – linker – strep tag – linker – strep tag – HIS tag)	MELLILKANAITTILTAVTFCFASGQNITEEFYQSTCSAV SKGYLSALRTGWYTSVITIELSNIKENKCNGTDAKVKLI KQELDKYKNAVTELQLLMQSTPATNNRARRELPRFMN YTLNNAKKTNVTLSKKRKRRFLGFLLGVGSAIASGVA VCKVLHLEGEVNKIKSALLSTNKAVVSLSNGVSVLTFK VLDLKNYIDKQLLPILNKQSCSISNIETVIEFQQKNNRLL EITREFSVNAGVTTPVSTYMLTNSELLSLINDMPITNDQ KKLMSNNVQIVRQQSYSIMCIIKEEVLAYVVQLPLYGVI DTPCWKLHTSPLCTTNTKEGSNICLTRTDRGWYCDNA GSVSFFPQAETCKVQSNRVFCDTMNSLTLPSEVNLCNV DIFNPKYDCKIMTSKTDVSSSVITSLGAIVSCYGKTKCT ASNKNRGIIKTFSNGCDYVSNKGVDTVSVGNTLYYVN KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN

SEQ	Name	Sequence
		QSLAFIRKSDELLGGSGGSGGGGSGGSGGENQKLIANQ FNSAIGKIQDSLSSTASALGKLQDVVNQNAQALNTLVK QLSSNFGAISSVLGGSGGSGGDVDLGDISGINASVVNIQ KEIDRLNEVAKNLNESGGSGGSLVPRGGSAGSGWSHP QFEKGGGSGGGSGGGSWSHPQFEKGSKGGHHHHHH
29	HR1 (HRA) from RSV	FLGFLLGVGSAIASGVAVCKVLHLEGEVNKIKSALLST NKAVVSLSNGVSVLTFKVLDLKNYIDKQLLPILNKQSC SIS
30	HR2 (HRB) from RSV	KQEGKSLYVKGEPIINFYDPLVFPSDEFDASISQVNEKIN QSLAFIRKSDELL

CLAIMS

1. A fusion protein comprising an ectodomain of a viral fusion protein linked to a heptad repeat (HR) from a SARS-COV-2 spike (S) protein or a HR from respiratory syncytial virus (RSV) F protein.

2. A fusion protein comprising an ectodomain of a viral fusion protein linked to a heptad repeat (HR) from a respiratory syncytial virus (RSV) F protein.

3. The fusion protein of claim 1 or claim 2, wherein the HR is HR1 from RSV F protein.

4. The fusion protein of claim 1 or 2 comprising the ectodomain linked to two HRs from a RSV F protein.

5. The fusion protein of claim 4 comprising a HR1 and HR2 from the RSV F protein.

6. The fusion protein of any one of claims 1 to 5, wherein the HR(s) are additional to any HRs in the ectodomain.

7. The fusion protein of any one of claims 1 to 6, wherein the ectodomain lacks a transmembrane region and cytoplasmic domain.

8. The fusion protein of any one of claims 1 to 7, wherein the ectodomain is a respiratory syncytial virus F protein ectodomain.

9. The fusion protein of any one of claims 1 to 8, wherein the ectodomain comprises one or more mutations to stabilize the ectodomain in a prefusion conformation.

10. The fusion protein of claim 9, wherein the mutation(s) introduce one or more cysteine residues that form a disulfide bond that is not present in the native ectodomain and/or a mutation that introduces an amino acid that fills a hydrophobic cavity that is present in the native ectodomain.

11. The fusion protein of claim 9 or 10, comprising one or more of the following groups of mutations that stabilize the F protein ectodomain in a prefusion conformation:
(i) S155C, S290C, S190F and V207L relative to SEQ ID NO: 3;
(ii) N67I and S215P relative to SEQ ID NO: 3;
(iii) N67I, S215P and E487Q relative to SEQ ID NO: 3;
(iv) D486H, E487Q, F488W and D489H relative to SEQ ID NO: 3.

12. The fusion protein of claim 11 comprising respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3 or comprises or consists of a sequence set forth in SEQ ID NO: 18.

13. The fusion protein of claim 12 comprising in amino to carboxy order:
(i) respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3, HR1 from RSV and HR2 from RSV; or
(ii) respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3, HR2 from RSV and HR1 from RSV.

14. The fusion protein of any one of claims 1 to 13, comprising a linker positioned between the ectodomain and the HR.

15. The fusion protein of claim 14, wherein the linker comprises glycine and serine.

16. The fusion protein of claim 14 or 15, wherein the linker is selected from a sequence set forth in any one of SEQ ID NOs: 10 to 13.

17. The fusion protein of any one of any one of claims 1 to 16 comprising in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3 or comprising or consisting of the sequence set forth in SEQ ID NO: 18, a linker; HR1 from RSV; and HR2 from RSV.

18. The fusion protein of any one of any one of claims 1 to 16 comprising in amino to carboxy order respiratory syncytial virus F protein ectodomain comprising the

following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3 or comprising or consisting of the sequence set forth in SEQ ID NO: 18, a linker comprising the sequence set forth in SEQ ID NO: 13; HR1 from RSV and HR2 from RSV.

19. A fusion protein comprising an ectodomain of a viral fusion protein linked to a heptad repeat (HR) from a SARS-COV-2 spike (S) protein.

20. The fusion protein of claim 19, wherein the HR is HR1 from SARS-COV-2 S protein.

21. The fusion protein of claim 20 comprising the ectodomain linked to two HRs from a SARS-COV-2 S protein.

22. The fusion protein of claim 21 comprising a HR1 and HR 2 from the SARS-COV-2 S protein.

23. The fusion protein of any one of claims 19 to 22, wherein the HR(s) are additional to any HRs in the ectodomain.

24. The fusion protein of any one of claims 19 to 23 wherein the ectodomain lacks a transmembrane region and cytoplasmic domain.

25. The fusion protein of any one of claims 19 to 24, wherein the ectodomain comprises one or more mutations to stabilize the ectodomain in a prefusion conformation.

26. The fusion protein of any one of claims 19 to 25, wherein the ectodomain is a respiratory syncytial virus F protein ectodomain.

27. The fusion protein of claim 26, comprising one or more of the following groups of mutations that stabilize the F protein ectodomain in a prefusion conformation:

(i) S155C, S290C, S190F and V207L relative to SEQ ID NO: 3;

(ii) N67I and S215P relative to SEQ ID NO: 3;

(iii) N67I, S215P and E487Q relative to SEQ ID NO: 3;

(iv) D486H, E487Q, F488W and D489H relative to SEQ ID NO: 3.

28. The fusion protein of claim 26 comprising respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3 or comprising or consisting of the sequence set forth in SEQ ID NO: 18.

29. The fusion protein of claim 28 comprising in amino to carboxy order:
(i) respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3 or comprising or consisting of the sequence set forth in SEQ ID NO: 18, HR1 and HR2; or
(ii) respiratory syncytial virus F protein ectodomain comprising the following mutations S155C, S290C, S190F and V207L relative to SEQ ID NO: 3 or comprising or consisting of the sequence set forth in SEQ ID NO: 18, HR2 and HR1.

30. The fusion protein of any one of claims 19 to 25, wherein the ectodomain is a SARS-COV-2 S protein ectodomain.

31. The fusion protein of claim 30, comprising one or more of the following:
(i) K986P and V987P relative to SEQ ID NO: 4; and/or
(ii) mutation of the furin cleavage site at positions 682 to 685 of SEQ ID NO: 4.

32. The fusion protein of any one of claims 19 to 25, comprising the SARS-COV-2 S protein ectodomain and one HR of a SARS-COV-2 S protein.

33. The fusion protein of claims 32, wherein the HR is HR1.

34. The fusion protein of any one of claims 30 to 33, comprising in amino to carboxy terminal order the SARS-COV-2 S protein ectodomain and HR1 of a SARS-COV-2 S protein.

35. The fusion protein of any one of any one of claims 1 to 34, wherein the ectodomain and the HR(s) are linked by a linker.

36. The fusion protein of claim 35, wherein the linker comprises the sequence $(GGGGS)_2$ or $(GGGGS)_3$.

37. A nucleic acid encoding the fusion protein of any one of claims 1 to 36.

38. The nucleic acid of claim 37, which is a RNA.

39. The nucleic acid of claim 38, which is within a mRNA or sa-mRNA vaccine.

40. A nanoparticle comprising the nucleic acid of any one of claims 37 to 39.

41. A composition comprising the fusion protein of any one of claims 1 to 36, the nucleic acid of any one of claims 37 to 39 or the nanoparticle of claim 40.

42. The composition of claim 41 additionally comprising an adjuvant.

43. The composition of claim 42, wherein the adjuvant comprises an oil-in-water emulsion of a squalene, polyoxyethylene sorbitan monooleate and sorbitan trioleate compounds.

44. A method of inducing an immune response in a subject, the method comprising administering the fusion protein of any one of claims 1 to 36, the nucleic acid of any one of claims 37 to 39 or the nanoparticle of claim 40 or the composition of any one of claims 41 to 43 to the subject.

45. A method of immunizing a subject, the method comprising administering the fusion protein of any one of claims 1 to 36, the nucleic acid of any one of claims 37 to 39 or the nanoparticle of claim 40 or the composition of any one of claims 41 to 43 to the subject.

46. A method of treating or preventing an infection by a virus, the method comprising administering the fusion protein of any one of claims 1 to 36, the nucleic acid of any one of claims 37 to 39 or the nanoparticle of claim 40 or the composition of any one of claims 41 to 43 to the subject.

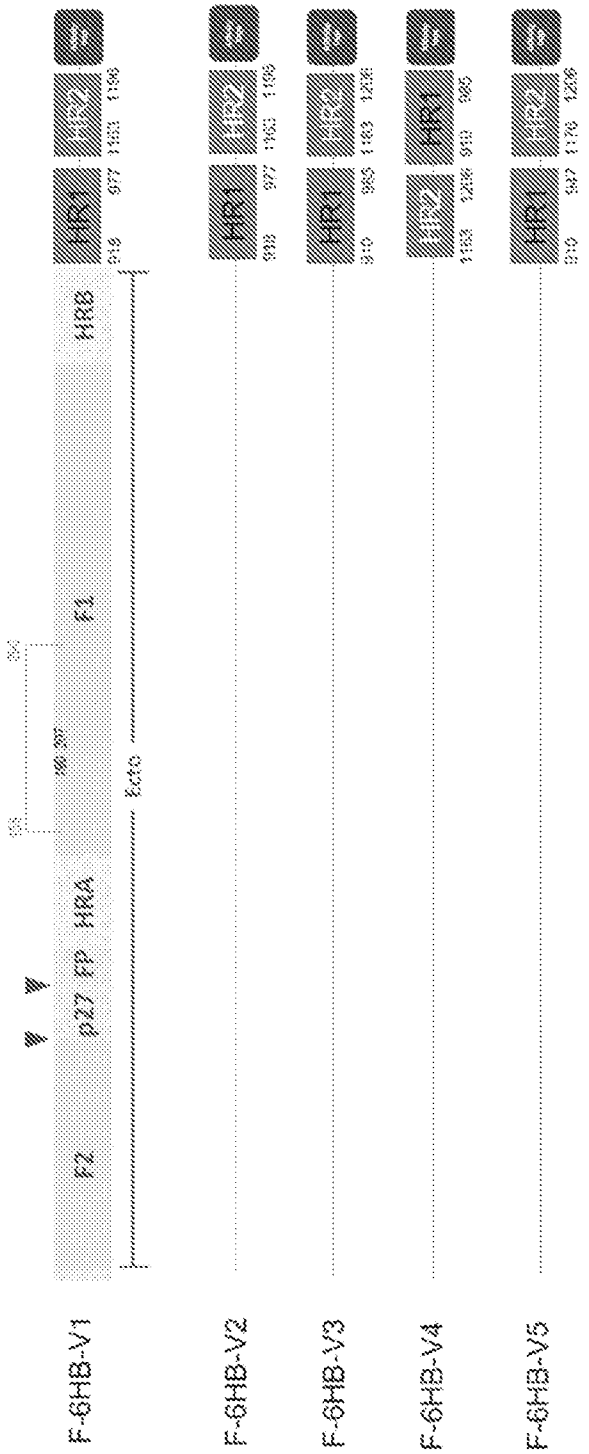
F2
p27
FP
HRA
F1
HRB
Ecto
HR1
HR2
F-6HB-V1
F-6HB-V2
F-6HB-V3
F-6HB-V4
F-6HB-V5

FIGURE 1

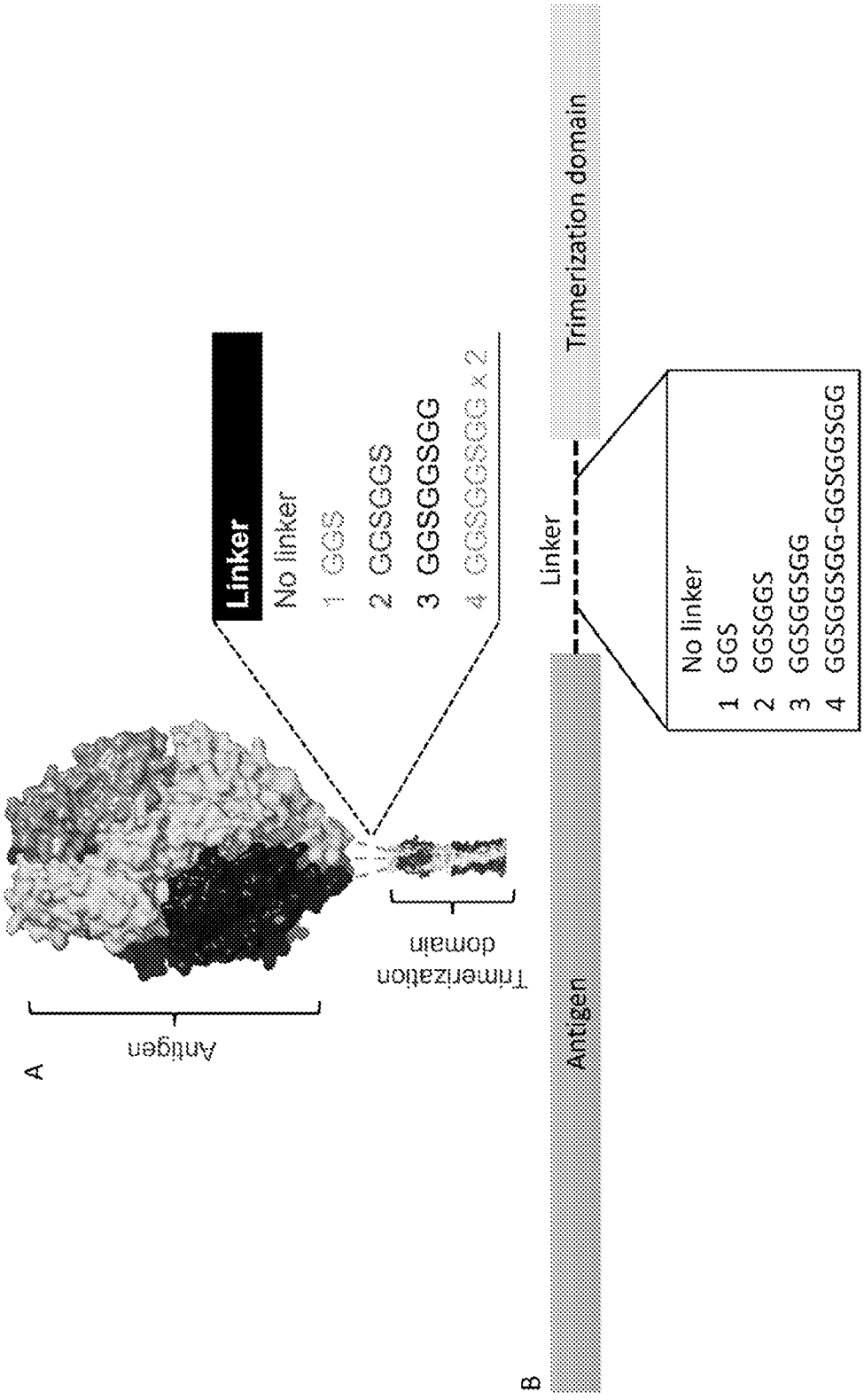
A
Antigen
Trimerization domain
Linker
No linker
1 GGS
2 GGSGGS
3 GGSGGSGGG
4 GGSGGSGGG x 2
B
Antigen
Linker
Trimerization domain
No linker
1 GGS
2 GGSGGS
3 GGSGGSGGG
4 GGSGGSGG-GGSGGSGG

FIGURE 2

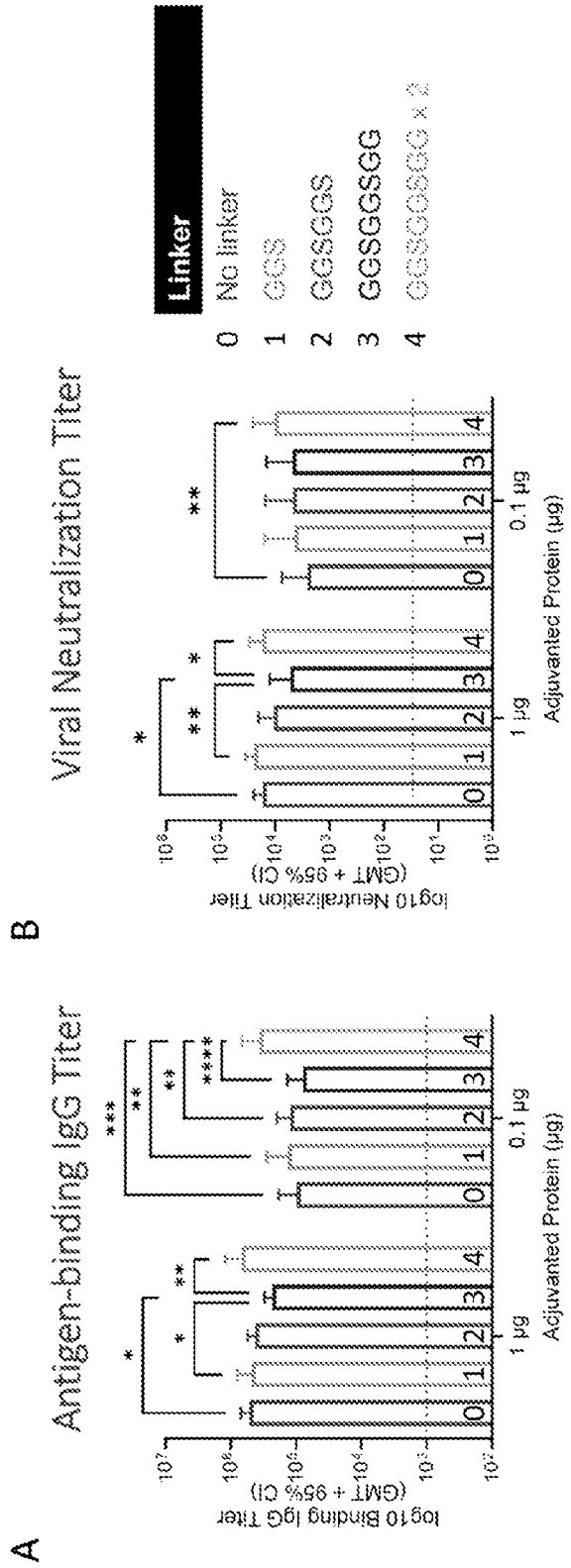
A
Antigen-binding IgG Titer
log10 Binding IgG Titer (GMT + 95% CI)
Adjuvanted Protein (µg)
1 µg
0.1 µg
B
Viral Neutralization Titer
log10 Neutralization Titer (GMT + 95% CI)
Linker
0 No linker
1 GGS
2 GGSGGS
3 GGSGGSGG
4 GGSGGSGG x 2

FIGURE 3

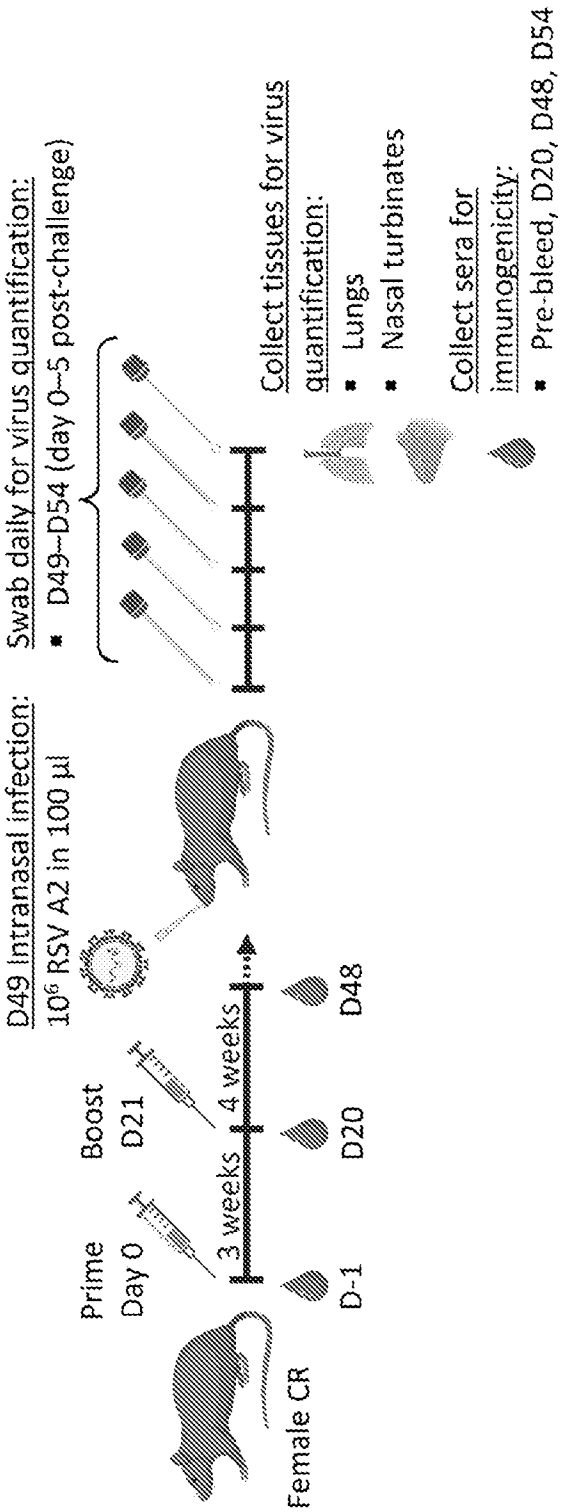
Female CR
Prime
Day 0
Boost
D21
3 weeks
4 weeks
D-1
D20
D48
D49 Intranasal infection:
10⁶ RSV A2 in 100 µl
Swab daily for virus quantification:
D49–D54 (day 0–5 post-challenge)
Collect tissues for virus quantification:
Lungs
Nasal turbinates
Collect sera for immunogenicity:
Pre-bleed, D20, D48, D54

FIGURE 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB23/61303

A. CLASSIFICATION OF SUBJECT MATTER

IPC - INV. A61K 39/12; A61K 39/155; A61K 39/42; C07K 14/005 (2023.01)
ADD. A61K 39/00 (2023.01)

CPC - INV. A61K 39/12; A61K 39/155; A61K 39/42; C07K 14/005
ADD. A61K 39/00; C07K 2319/00; C07K 2319/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic database consulted during the international search (name of database and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2022/038504 A1 (GRIFOLS DIAGNOSTIC SOLUTIONS INC. et. al) 24 February 2022; pg 11, paragraph 1; page 13, paragraphs 2-3.	1, 3/1, 19-22 --- 4/1, 5/4/1, 23
X --- Y	WO 2012/158613 A1 (NOVARTIS AG et. al) 22 November 2012; paragraphs [004], [021]	2, 3/2, 4/2, 5/4/2, --- 4/1, 5/4/1
Y	COLL. "Heptad-repeat sequences in the glycoprotein of rhabdoviruses" 107-114. Virus Genes. Web. June 1995; Abstract; DOI: 10.1007/BF01702591	23

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
09 January 2024 (09.01.2024)	MAR 05 2024
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB23/61303

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed.

   b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)),

      ☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB23/61303

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-18, 24-46
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.