CLAIMS

~~What is claimed is:~~

1. A modified nucleic acid molecule encoding a modified spike protein derived from a SARS-CoV-2 spike protein comprising one or more amino acid substitutions of asparagine (N) to glutamine (Q) at N-linked glycosylation sequons (N-X-S/T), wherein X is any amino acid residue except proline, and S/T denotes a serine or threonine residue, wherein the spike protein further comprises: one or more amino acid deletions or additions at N-linked glycosylation sequons (N-X-S/T) to eliminate N-linked glycan sequons, or one or more amino acid substitutions of S/T to alanine (A) at O-linked glycosylation sites to eliminate O-linked glycosylation sites, wherein the modified nucleic acid molecule is an mRNA or a double-strand or single-strand DNA.

~~2. The modified nucleic acid molecule of claim 1, which comprises one or more amino acid deletions or additions at N-linked glycosylation sequons (N-X-S/T) to eliminate N-linked glycan sequons.~~

~~3. The modified nucleic acid molecule of claim 1, which comprises one or more amino acid substitutions of S/T to alanine (A) at O-linked glycosylation sites to eliminate O-linked glycosylation sites.~~

~~4. The modified nucleic acid molecule, which is an mRNA or a double-strand or single-strand DNA.~~

~~5. The modified nucleic acid molecule of any of claims 1 to 4, wherein the modified spike protein is derived from a SARS-CoV-2 spike protein.~~

~~6.~~2. The modified nucleic acid molecule of ~~any of claims 1 to 4~~claim 1, wherein the spike protein comprises an amino acid sequence of SEQ ID NO: 2, 16, 18 or 20, or amino acid sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 2, 16, 18 or 20.

~~7.~~3. The modified nucleic acid molecule of claim ~~6~~2, wherein the nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 2, 16, 18 or 20 is an mRNA comprising the nucleotide sequence of SEQ ID NO: 1, 15, 17 or 19 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 1, 15, 17 or 19 respectively.

~~8.~~4. The modified nucleic acid molecule of ~~any of claims 1 to 4~~claim 1, wherein the modified spike protein comprises an amino acid sequence of SEQ ID NO: 4, 22, 24 or 26, wherein the modified spike protein comprises a receptor binding domain (RBD) lacking glycosylation sites.

~~9.~~5. The modified nucleic acid molecule of claim ~~8~~4, wherein the modified nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 4, 22, 24 or 26 comprises the nucleotide sequence of SEQ ID NO: 3, 21, 23 or 25 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 3, 21, 23 or 25 respectively.

~~10.~~6. The modified nucleic acid molecule of ~~any of claims 1 to 4~~claim 1, wherein the modified spike protein comprises an amino acid sequence of SEQ ID NO: 6, 28, 30 or 32, wherein the modified spike protein comprises a S2 subunit lacking glycosylation sites.

~~11.~~7. The modified nucleic acid molecule of claim ~~10~~6, wherein the modified nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 6, 28, 30 or 32 comprises the nucleotide sequence of SEQ ID NO: 5, 27, 29 or 31 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 527, 29 or 31 respectively.

~~12.~~8. The modified nucleic acid molecule of ~~any of claims 1 to 4~~claim 1, wherein the modified spike protein described herein comprises an amino acid sequence of SEQ ID NO: 8 or 34, wherein the modified spike protein comprises a S2 subunit that consists of a single glycosylation site~~. In some embodiments~~, wherein the single glycosylation site is at the position N1194.

~~13.~~9. The modified nucleic acid molecule of claim ~~12~~8, wherein the modified nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 8 or 34 comprises the nucleotide sequence of SEQ ID NO: 7 or 33 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 7 or 33 respectively.

~~14.~~10. The modified nucleic acid molecule of ~~any of claims 1 to 4~~claim 1, wherein the modified spike protein comprises an amino acid sequence of SEQ ID NO: 10 or 36, wherein the modified spike protein comprises a receptor binding domain (RBD) lacking glycosylation sites, and an amino acid substitution of N801 to Q801.

~~15.~~11. The modified nucleic acid molecule of claim ~~14~~10, wherein the modified nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 10 or 36 comprises the nucleotide sequence of SEQ ID NO: 9 or 35 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 9 or 35 respectively.

~~16.~~12. The modified nucleic acid molecule of ~~any of claims 1 to 4~~claim 1, wherein the modified spike protein comprises an amino acid sequence of SEQ ID NO: 12 or 38, wherein the modified spike protein comprises a receptor binding domain (RBD) lacking glycosylation sites, and an amino acid substitution of N1194 to Q1194.

~~17.~~13. The modified nucleic acid molecule of claim ~~16~~12, wherein the modified nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 12 or 38 comprises the nucleotide sequence of SEQ ID NO: 11 or 37 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 11 or 37 respectively.

~~18.~~14. The modified nucleic acid molecule of ~~any of claims 1 to 4~~claim 1, wherein the modified spike protein described herein comprises an amino acid sequence of SEQ ID NO: 14 or 40, wherein the modified spike protein comprises a modified receptor binding domain (RBD) lacking glycosylation sites, and amino acid substitutions of N122 to Q122, N165 to Q165, and N234 to Q234.

~~19.~~15. The modified nucleic acid molecule of claim ~~18~~14, wherein the modified nucleic acid molecule encoding the amino acid sequence of SEQ ID NO: 14 or 40 comprises the nucleotide sequence of SEQ ID NO: 13 or 39 respectively, or a nucleotide sequence having at least about 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13 or 39 respectively.

~~20. The modified nucleic acid molecule of any of claims 1 to 4, wherein the modified spike protein comprises a S1 subunit lacking glycosylation sites.~~

~~21. The modified nucleic acid molecule of any of claims 1 to 4, wherein the modified spike protein comprises both S1 and S2 subunits lacking glycosylation sites.~~

~~22.~~16. The modified nucleic acid molecule of ~~any of claims 1 to 4~~claim 1, wherein the mRNA for S-(deg-RBD) has the sequence of SEQ ID NO: 3, 21, 23 or 25, the mRNA or DNA for S-(deg-S2) has the sequence of SEQ ID NO: 5, 27, 29 or 31, the mRNA or DNA for S-(deg-S2-1194) has the sequence of SEQ ID NO: 7 or 33, the mRNA or DNA for S-(deg-RBD-801) has the sequence of SEQ ID NO: 9 or 35, the mRNA or DNA for S-(deg-RBD-1194) has the sequence of SEQ ID NO: 11 or 37, the mRNA or DNA for S-(deg-RBD-122-165-234) has the sequence of SEQ ID NO: 13 or 39.

~~23.~~17. The modified nucleic acid molecule of any of claims 1 to ~~22~~16, which can be used as a coronavirus mRNA vaccine.

~~24. The modified nucleic acid molecule of any of claims 1 to 23, wherein immunization of the modified nucleic acid molecule causes the upregulation of BiP/GRP78, XBP1 and p-eIF2α to induce cell apoptosis and CD8 T-cell response.~~

~~25. The modified nucleic acid molecule of any of claims 1 to 23, wherein the coronavirus (CoV) is selected from the group consisting of alpha-CoV, beta-CoV, gamma-CoV, delta-CoV2, and omicron-CoV2.~~

~~26.~~18. A combo vaccine, comprising the mRNA vaccine of claim ~~23~~ 17 and one or more additional vaccines selected from the group consisting of one or more COVID-19 vaccine, influenza (flu) vaccine, advenovirus vaccine, anthrax vaccine, cholera vaccine, diphtheria

vaccine, hepatitis A or B vaccine, HPV vaccine, measle vaccine, mumps vaccine, smallpox vaccine, rotavirus vaccine, tuberculosis vaccine, pneumoccal vaccine and Haemophilus influenzae type b vaccine and any combination thereof.

~~27. The combo vaccine of claim 26, wherein the additional vaccine is selected from one or more COVID-19 vaccine, influenza (flu) vaccine, advenovirus vaccine, anthrax vaccine, cholera vaccine, diphtheria vaccine, hepatitis A or B vaccine, HPV vaccine, measle vaccine, mumps vaccine, smallpox vaccine, rotavirus vaccine, tuberculosis vaccine, pneumoccal vaccine and Haemophilus influenzae type b vaccine and any combination thereof.~~

~~28. A nanoparticle, which is guanidine based and used as carrier for delivering the modified nucleic acid molecule of any one of claims 1 to 25 to a subject.~~

~~29. The nanoparticle of claim 28, which is a liposome or polymersome.~~

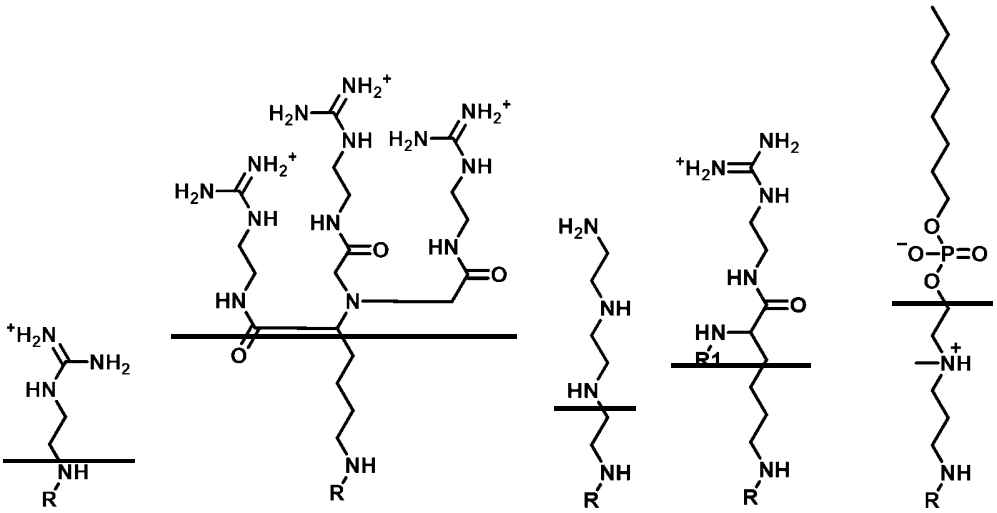
~~30. An mRNA nanocluster comprising the vaccine of claim 26 or 27 formulated in a lipid nanoparticle.~~

~~31. The mRNA nanocluster of claim 30, wherein the lipid nanoparticle is a biodegradable lipid nanoparticle.~~

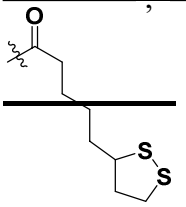
~~32. The mRNA nanocluster of claim 30 or 31, wherein the lipid nanoparticle is guanidine-based polymers.~~

~~33. An mRNA nanocluster, comprising a lipid nanoparticle encapsulated with the mRNA vaccine, wherein the biodegradable lipid nanoparticle comprises guanidine-based and zwitterionic units, wherein the guanidine based as well as zwitterionic groups attach to a lipid tail, and wherein the guanidine based groups adhere to mRNA, thereby forming salt bridges between the guanidinium groups and the phosphates in the mRNA.~~

~~34. The nanoparticle of claim 28 or 29 or the mRNA nanocluster of any one of claims 30 to 33, wherein the guanidine based polymers is P1, P2, P3, Pb or Pz:~~



P1 , P2 , P3 , Pb and Pz , wherein R is

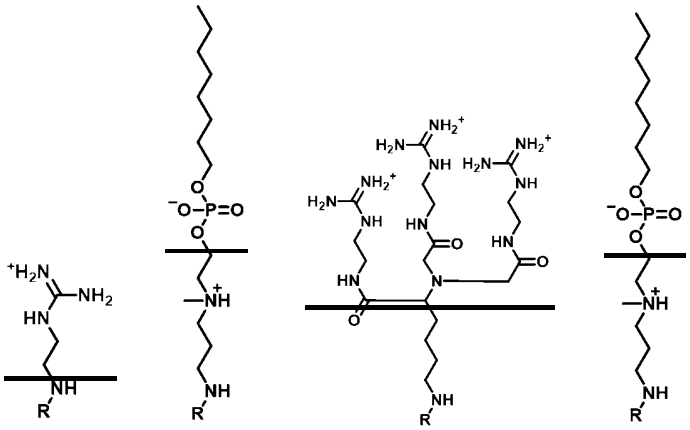


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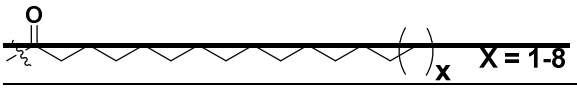
35. The nanoparticle of claim 28 or 29 or the mRNA nanocluster of any one of claims 30 to 34, wherein the guanidine based polymer forms a copolymer such as P1/P3 copolymer, P2/P3 copolymer, P1/Pb copolymer, P2/Pb copolymer, P1/Pz copolymer and P2/Pz copolymer.

36. The nanoparticle of claim 28 or 29 or the mRNA nanocluster of any one of claims 30 to 35, wherein the guanidine-based and zwitterionic nanoparticles are a mixture of P1, and/or

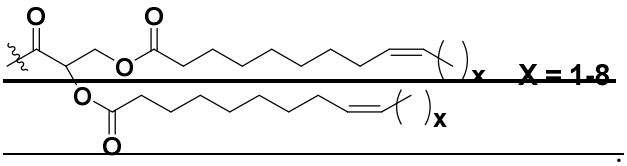
~~P2 and Pz~~



~~, where R =~~



~~or~~



~~37. The nanoparticle of claim 28 or 29 or the mRNA nanocluster of any one of claims 30 to 36, which has a nanoparticle/mRNA (N/P) ratio from about 1 to about 100.~~

~~38. The nanoparticle of claim 28 or 29 or the mRNA nanocluster of any one of claims 30 to 37, which has a nanoparticle/mRNA (N/P) ratio of about 10 or about 20.~~

~~39. A nanoparticle composition, comprising a nanoparticle attached with the mRNA vaccine of claim 23.~~

~~40.~~19. ~~A method of~~Use of the modified nucleic acid molecule according to any one of claims 1-17 in the manufacture of a composition for preventing or treating a coronavirus infection~~, comprising administering an effective amount of the modified nucleic acid molecule of any one of claims 1 to 25, the nanoparticle of claim 28 or 29, the mRNA nanocluster of any one of claims 30 to 38 or a nanoparticle composition of claim 39~~ in a subject infected with, or at risk of being infected with, a coronavirus.

~~41. A method of boosting an adaptive immune response, comprising administering an effective amount of the modified nucleic acid molecule of any one of claims 1 to 25, the nanoparticle of claim 28 or 29, the mRNA nanocluster of any one of claims 30 to 38 or a nanoparticle composition of claim 39 to a subject.~~

~~42. The method of claim 41 or 42, wherein the modified nucleic acid molecule of any one of claims 1 to 25, the nanoparticle of claim 28 or 29, the mRNA nanocluster of any one of claims 30 to 38 or a nanoparticle composition of claim 39 is administered in an initial dose and two, three or four booster doses.~~

~~43. The method of claim 41 or 42, wherein the modified nucleic acid molecule of any one of claims 1 to 25, the nanoparticle of claim 28 or 29, the mRNA nanocluster of any one of claims 30 to 38 or a nanoparticle composition of claim 39 is administered in an initial dose and in at least one booster dose about one month, about two months, about three months, about four months, about five months, or about six months following the initial dose.~~

~~44.~~20. The ~~method~~ use of claim ~~41 or 42~~19, wherein the composition is formulated as a combination comprising one or more additional vaccines, wherein each member of the combination comprising an effective amount of the modified nucleic acid molecule ~~the dose ranges~~ ranging from about 5 μg to about 50 μg ~~of the modified nucleic acid molecule of any one of claims 1 to 25~~.

~~45. The method of claim 41 or 42, wherein the dose is 30 μg.~~

~~46. The method of claim 41 or 42, wherein the modified nucleic acid molecule of any one of claims 1 to 25, the nanoparticle of claim 28 or 29, the mRNA nanocluster of any one of claims 30 to 38 or a nanoparticle composition of claim 39 is administered via intravenous route, intramuscular route, intradermal route, or subcutaneous route, or by infusion or nasal spray.~~

~~47. An immunogenic peptide, comprising at least one amino acid sequence selected from a group consisting of: TESIVRFPNITNL (SEQ ID NO: 41), NITNLCPFGEVFNATR (SEQ ID NO: 42), LYNSASFSTFK (SEQ ID NO: 43), LDSKVGGNYN (SEQ ID NO: 44),~~

~~KSNLKPFERDIST (SEQ ID NO: 45), KPFERDISTEIYQAG (SEQ ID NO: 46), GPKKSTNLVKNKC (SEQ ID NO: 47), NCDVVIGIVNNTVY (SEQ ID NO: 48), PELDSFKEELDKYFKNHTS (SEQ ID NO: 49), VNIQKEIDRLNEVA (SEQ ID NO: 50), NLNESLIDLQ (SEQ ID NO: 51) and LGKYEQYIKWP (SEQ ID NO: 52) or an amino acid sequence having at least about 99%, 98%, 97%, 96%, 95% or 90% identity to any of SEQ ID NOs: 41 to 52.~~

~~48. The immunogenic peptide of claim 47, comprising at least one amino acid sequence selected from a group consisting of SEQ ID NOs: 41 to 43 and 45 to 51.~~

~~49. An immunogenic composition, comprising an immunogenic peptide of claim 47 or 48.~~