NPs) also demonstrated superior efficacy of multilamellar tumor specific RNA-NPs, relative to anionic LPX and RNA LPX, and demonstrated the ability to systemically activate DCs, induce antigen specific immunity, and elicit anti-tumor efficacy *in vivo*. The administration of multilamellar RNA NPs to diseased animals (e.g., tumor bearing mice) also led to increased survival of these animals. Without being bound to a particular theory, the multilamellar RNA nanoparticles of the present disclosure are capable of safely and effectively inducing immunity against SARS-CoV-2.

**[0006a]** In one aspect, the present disclosure provides a nanoparticle comprising a positively-charged surface and an interior comprising (i) a core and (ii) at least two nucleic acid layers comprising mRNA encoding a SARS-CoV-2 protein, wherein each nucleic acid layer is positioned between a cationic lipid bilayer, wherein the nanoparticle does not comprise a neutral lipid.

**[0006b]** In another aspect, the present disclosure provides a method of making a nanoparticle comprising a positively-charged surface and an interior comprising (i) a core and (ii) at least two nucleic acid layers comprising mRNA molecules encoding a SARS-CoV-2 protein, wherein each nucleic acid layer is positioned between a cationic lipid bilayer, wherein the nanoparticle does not comprise a neutral lipid, said method comprising: (A) mixing nucleic acid molecules encoding a SARS-CoV-2 protein and liposomes at a nucleic acid: liposome ratio of about 1 to about 5 to about 1 to about 20, optionally, about 1 to about 15, to obtain a nucleic acid-coated liposomes, wherein the liposomes are made by a process of making liposomes comprising drying a lipid mixture comprising a cationic lipid and an organic solvent by evaporating the organic solvent under a vacuum; and (B) mixing the nucleic acid-coated liposomes with a surplus amount of liposomes.

**[0006c]** In another aspect, the present disclosure provides a cell comprising a nanoparticle as described in the disclosure.

**[0006d]** In another aspect, the present disclosure provides a pharmaceutical composition comprising a plurality of nanoparticles according to the disclosure and a pharmaceutically acceptable carrier, diluent, or excipient.

**[0006e]** In another aspect, the present disclosure provides a use of the pharmaceutical composition of the disclosure in the manufacture of a medicament for treating SARS-CoV-2 infection in a subject, wherein the medicament is to be administered to a subject, and wherein the administration induces an immune response against a SARS-CoV-2 virus in the subject.

**[0006f]** In another aspect, the present disclosure provides a use of the pharmaceutical composition of the disclosure in the manufacture of a medicament for preventing SARS-CoV-2 infection in a subject, wherein the medicament induces an immune response against SAR-CoV-2 in a subject, and wherein the medicament is to be administered to a subject.

**[0007]** The present disclosure provides a nanoparticle comprising a positively-charged surface and an interior comprising (i) a core and (ii) at least two nucleic acid layers, wherein each nucleic acid layer is positioned between a cationic lipid bilayer, wherein the nanoparticle comprises RNA molecules encoding a SARS-CoV-2 protein, optionally, a SARS-CoV-2 Spike (S) protein. In exemplary examples, the nanoparticle comprises at least three nucleic acid layers, each of which is positioned between a cationic lipid bilayer. In exemplary examples, the nanoparticle comprises at least four or five or more nucleic acid layers, each of which is positioned between a cationic lipid bilayer. In various examples, the outermost layer of the nanoparticle comprises a cationic lipid bilayer. In various instances, the surface comprises a plurality of hydrophilic moieties of the cationic lipid of the cationic lipid bilayer. In exemplary examples, the core comprises a cationic lipid bilayer. Optionally, the core comprises less than about 0.5 wt% nucleic acid. The diameter of the nanoparticle, in various examples, is about 50 nm to about 250 nm in diameter, optionally, about 70 nm to about 200 nm in diameter. In exemplary instances, the nanoparticle is characterized by a zeta potential of about +40 mV to about +60 mV, optionally, about +45 mV to about +55 mV. The nanoparticle in various instances, has a zeta potential of about 50 mV. In some examples, the nucleic acid molecules are present at a nucleic acid molecule:cationic lipid ratio of about 1 to about 5 to about 1 to about 25, such as about 1 to about 5 to about 1 to about 20, optionally, about 1 to about 15, about 1 to about 10, or about 1 to about 7.5. In various examples, the nucleic acid molecules are RNA molecules, optionally, messenger RNA (mRNA).

**[0008]** In cancer-related examples of the technology described herein, the mRNA is *in vitro* transcribed mRNA wherein the *in vitro* transcription template is cDNA made from RNA extracted from a sample.

**[0009]** The present disclosure also provides a method of making a nanoparticle comprising a positively-charged surface and an interior comprising (i) a core and (ii) at least two nucleic acid layers, wherein each nucleic acid layer is positioned between a cationic lipid bilayer, wherein the nanoparticle comprises RNA molecules encoding a SARS-CoV-2 protein, optionally, a SARS-CoV-2 Spike (S) protein, said method comprising: (A) mixing nucleic acid molecules and liposomes at a RNA: liposome ratio of 1 to about 5 to about 1 to about 25, such as about 1 to about 5 to about 1 to about 20, optionally, about 1 to about 15, about 1 to about 10, or about 1 to about 7.5, to obtain a RNA-coated liposomes, wherein the liposomes are made by a process of making liposomes comprising drying a lipid mixture comprising a cationic lipid and an organic solvent by evaporating the organic solvent under a vacuum; and (B) mixing the RNA-coated liposomes with a surplus amount of liposomes. In exemplary examples, the lipid mixture comprises the cationic lipid and the organic solvent at a ratio of about 40 mg cationic lipid per mL organic solvent to about 60 mg cationic lipid per mL organic

**WHAT IS CLAIMED:**

1. A nanoparticle comprising a positively-charged surface and an interior comprising (i) a core and (ii) at least two nucleic acid layers comprising mRNA encoding a SARS-CoV-2 protein, wherein each nucleic acid layer is positioned between a cationic lipid bilayer, wherein the nanoparticle does not comprise a neutral lipid.
2. The nanoparticle of claim 1, comprising at least three, at least four, or at least five nucleic acid layers, each of which is positioned between a cationic lipid bilayer.
3. The nanoparticle of any one of claims 1-2, wherein the outermost layer of the nanoparticle comprises a cationic lipid bilayer.
4. The nanoparticle of any one of claims 1-3, wherein the core comprises a cationic lipid bilayer.
5. The nanoparticle of any one of claims 1-4, wherein the core comprises less than 0.5 wt% nucleic acid.
6. The nanoparticle of any one of claims 1-5, wherein the diameter of the nanoparticle is 50 nm to 500 nm in diameter, optionally, 70 nm to 200 nm in diameter.
7. The nanoparticle of any one of claims 1-6, comprising a zeta potential of 40 mV to 60 mV, optionally, 45 mV to 55 mV.
8. The nanoparticle of claim 7, comprising a zeta potential of 50 mV.
9. The nanoparticle of any one of claims 1-8, comprising nucleic acid molecules and cationic lipid at a ratio of 1 to 5 to 1 to 20, optionally, 1 to 15 or 1 to 7.5.
10. The nanoparticle of any one of claims 1-9, wherein the cationic lipid is DOTAP or DOTMA.
11. The nanoparticle of any one of claims 1-10, wherein the mRNAs encode a SARS-CoV-2 Spike (S) protein.
12. The nanoparticle of any one of claims 1-10, wherein the mRNA encodes one or more amino acid sequences selected from the group consisting of SEQ ID NOs: 1-13 or SEQ ID NOs: 23-27.
13. The nanoparticle of any one of claims 1-12, wherein the mRNAs encode a SARS-CoV-2 membrane protein, envelope protein, or a nucleocapsid protein.
14. A method of making a nanoparticle comprising a positively-charged surface and an interior comprising (i) a core and (ii) at least two nucleic acid layers comprising mRNA molecules encoding a SARS-CoV-2 protein, wherein each nucleic acid layer is positioned between a cationic lipid bilayer, wherein the nanoparticle does not comprise a neutral lipid, said method comprising:

    (A) mixing nucleic acid molecules comprising mRNA encoding a SARS-CoV-2 protein and liposomes at a nucleic acid: liposome ratio of 1 to 5 to 1 to 20, optionally, 1 to 15, to obtain a nucleic acid-coated liposomes, wherein the liposomes are made by a process of making liposomes comprising

drying a lipid mixture comprising a cationic lipid and an organic solvent by evaporating the organic solvent under a vacuum; and

(B) mixing the nucleic acid-coated liposomes with a surplus amount of liposomes.

15. The method of claim 14, wherein the lipid mixture comprises the cationic lipid and the organic solvent at a ratio of 40 mg cationic lipid per mL organic solvent to 60 mg cationic lipid per mL organic solvent, optionally, at a ratio of 50 mg cationic lipid per mL organic solvent.
16. The method of claim 14 or claim 15, wherein the process of making liposomes further comprises rehydrating the lipid mixture with a rehydration solution to form a rehydrated lipid mixture and then agitating, resting, and sizing the rehydrated lipid mixture.
17. The method of any one of claims 14-16, wherein the mRNAs encode a SARS-CoV-2 Spike (S) protein.
18. The method of any one of claims 14-16, wherein the mRNA encodes one or more amino acid sequences selected from the group consisting of SEQ ID NOs: 1-13 or 23-27.
19. The method of any one of the claims 14-18, wherein the mRNAs encode a SARS-CoV-2 membrane protein, envelope protein, or a nucleocapsid protein.
20. A cell comprising a nanoparticle as described in any one of claims 1-13.
21. The cell of claim 20, which is an antigen presenting cell (APC), optionally, a dendritic cell (DC).
22. A pharmaceutical composition comprising a plurality of nanoparticles according to any one of claims 1-13 and a pharmaceutically acceptable carrier, diluent, or excipient.
23. The pharmaceutical composition of claim 22, wherein the composition comprises $10^{10}$ nanoparticles per mL to $10^{15}$ nanoparticles per mL, optionally $10^{12}$ nanoparticles ± 10% per mL.
24. Use of the pharmaceutical composition of claim 22 or claim 23 in the manufacture of a medicament for preventing SARS-CoV-2 infection in a subject, wherein the medicament induces an immune response against SAR-CoV-2 in a subject, and wherein the medicament is to be administered to a subject.
25. Use of the pharmaceutical composition of claim 22 or claim 23 in the manufacture of a medicament for inducing an immune response against a SARS-CoV-2 virus in a subject, wherein the medicament is to be administered to a subject.
26. The use of claims 24 or 25, wherein a single dose of the composition comprises 0.00050 mg/kg to 1.5 mg/kg of nucleic acid.
27. The use of claim 26, wherein the nucleic acid is encapsulated into 0.008 mg/kg to 1.5 mg/kg of liposome material.
28. The use of claim 26 or claim 27, wherein a single dose of composition comprises 0.000625 mg/kg to 0.08 mg/kg nucleic acid.
29. The use of claim 28, wherein a single dose of composition comprises 0.000625 mg/kg of mRNA, 0.00125 mg/kg mRNA, 0.0025 mg/kg mRNA, 0.005 mg/kg mRNA, 0.01 mg/kg mRNA, 0.02 mg/kg mRNA, 0.04 mg/kg mRNA, or 0.08 mg/kg mRNA.
30. The use of any one of claims 24-29, wherein multiple doses of pharmaceutical composition is to be administered to a subject over a treatment period of 18 months.

31. The use of claim 30, wherein (a) an initial set of doses of the pharmaceutical composition is to be administered to a subject, and wherein each administration is separated by two weeks, followed by (b) a subsequent set of doses of pharmaceutical composition wherein each is to be administered once per month.
32. The use of claim 31, wherein the initial set of three doses are to be administered over an initial treatment period of four weeks, and the subsequent set of doses are to be administered over a subsequent treatment period of 12 months.