CLAIMS

We claim:

1. A process of encapsulating messenger RNA (mRNA) in lipid nanoparticles (LNPs) comprising a step of mixing (a) an mRNA solution comprising one or more mRNAs with (b) a lipid solution comprising one or more cationic lipids, one or more non-cationic lipids, and one or more PEG-modified lipids, to form mRNA encapsulated within LNPs (mRNA-LNPs) in a LNP formation solution, wherein the mRNA solution comprises less than 5 mM of citrate, and wherein the mRNA-LNPs have an encapsulation efficiency of greater than 60%.

2. The process of claim 1, wherein the mRNA solution and/or the lipid solution are at an ambient temperature prior to mixing, optionally wherein:
(i) the ambient temperature is less than about 35 °C, less than about 30 °C, less than about 26°C, less than about 23 °C, less than about 21 °C, less than about 20 °C, or less than about 18 °C; and/or
(ii) the ambient temperature ranges from about 18-32 °C, about 21-26 °C, or about 23-25 °C.

3. The process of any one of the preceding claims, wherein:
(i) the mRNA solution comprises less than about 4.0 mM of citrate, less than about 3.0 mM of citrate, less than about 2.5 mM of citrate, less than about 2.0 mM of citrate, less than about 1.5 mM of citrate, less than about 1.25 mM of citrate, less than about 1.0 mM of citrate, or less than about 0.5 mM of citrate; and/or
(ii) the mRNA solution comprises about 3.0 mM of citrate, about 2.5 mM of citrate, about 2.0 mM of citrate, about 1.5 mM of citrate, about 1.25 mM of citrate, about 1.0 mM of citrate, about 0.5 mM of citrate or 0 mM of citrate.

4. The process of claim 1 or 2, wherein the mRNA solution comprises at least 0.1 mM citrate, optionally wherein the mRNA solution comprises between about 1 mM and 4 mM citrate.

5. The process of claim 4, wherein the mRNA solution comprises between about 1 mM and 3 mM citrate, optionally wherein the mRNA solution comprises between about 1 mM and 2 mM citrate or between about 2 mM and 3 mM citrate.

6. The process of claim 4, wherein the mRNA solution comprises about 1 mM citrate, about 2 mM citrate or about 3 mM citrate.

7. The process of any one of the preceding claims, wherein:
(i) the mRNA solution further comprises trehalose; and/or
(ii) the process does not require a step of heating the mRNA solution and the lipid solution prior to the mixing step.

8. The process of any one of the preceding claims, wherein the mRNA solution comprises greater than about 1 g of mRNA per 12 L of the mRNA solution, optionally wherein:

the mRNA solution comprises about 1 g of mRNA per 8 L of the mRNA solution, about 1 g of mRNA per 4 L of the mRNA solution or about 1 g of mRNA per 2 L of the mRNA solution; or

the concentration of mRNA in the mRNA solution is greater than about 0.125 mg/mL, greater than about 0.25 mg/mL, greater than about 0.5 mg/mL, or greater than about 1.0 mg/mL.

9. The process of any one of the preceding claims, wherein the mRNA solution and the lipid solution are mixed at a ratio (v/v) of between 2:1 and 6:1, optionally wherein:
(i) the mRNA solution and the lipid solution are mixed at a ratio (v/v) of greater than about 3:1; or
(ii) the mRNA solution and the lipid solution are mixed at a ratio (v/v) of about 4:1.

10. The process of any one of the preceding claims, wherein:
(i) the mRNA solution has a pH between 3.0 and 5.0, optionally wherein the mRNA solution has a pH of about 3.5, 4.0, or 4.5; and/or
(ii) the mRNA solution comprises about 37.5 mM to 300 mM NaCl, optionally wherein the mRNA solution comprises about 37.5 mM, about 75 mM, about 100 mM, about 150 mM, or about 300 mM NaCl.

11. The process of claim 1 or 2, wherein the mRNA solution comprises about 2.5 mM citrate, about 150 mM NaCl, and pH of about 4.5.

12. The process of any one of the preceding claims, wherein the process further comprises a step of incubating the mRNA-LNPs, optionally wherein:

(i) the mRNA-LNPs are incubated at a temperature of between 21 °C and 65 °C, optionally wherein the mRNA-LNPs are incubated at a temperature of about 26 °C, about 30 °C, or about 65 °C; and/or

(ii) the mRNA-LNPs are incubated for greater than about 20 minutes, about 30 minutes, about 60 minutes, about 90 minutes, or about 120 minutes, optionally wherein the mRNA-LNPs are incubated for about 60 minutes.

13. The process of any one of the preceding claims, wherein:

(i) the lipid solution comprises less than 50%, less than 25%, less than 20%, less than 10%, less than 5% non-aqueous solvent, such as ethanol;

(ii) the lipid solution further comprises one or more cholesterol-based lipids;

(iii) the mRNA-LNPs are purified by Tangential Flow Filtration;

(iv) the mRNA-LNPs have an average size of less than 150 nm, less than 100 nm, less than 80 nm, less than 60 nm, or less than 40 nm, optionally wherein the mRNA-LNPs have an average size ranging from 40-70 nm;

(v) the lipid nanoparticles have a PDI of less than about 0.3, less than about 0.2, less than about 0.18, less than about 0.15, less than about 0.1;

(vi) the mRNA-LNPs have a N/P ratio of between 1 to 10, optionally wherein the mRNA-LNPs have a N/P ratio of between 2 to 6, further optionally wherein the mRNA-LNPs have a N/P ratio of about 4; and/or

(vii) the mRNA solution and the lipid solution are mixed by a pulse-less flow pump, optionally wherein the pump is a gear pump, further optionally wherein the pump is a centrifugal pump.

14. The process of any one of the preceding claims, wherein the encapsulation efficiency of the mRNA-LNPs is greater than about 65%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%;

15. The process of any one of the preceding claims, wherein 5g or more, 10 g or more, 20 g or more, 50 g or more, 100 g or more, or 1 kg or more of mRNA is encapsulated in lipid nanoparticles in a single batch.

16. The process of any one of the preceding claims, wherein:

(i) the mRNA solution is mixed at a flow rate ranging from about 150-250 ml/minute, 250-500 ml/minute, 500-1000 ml/minute, 1000-2000 ml/minute, 2000-3000 ml/minute, 3000-4000 ml/minute, or 4000-5000 ml/minute, optionally wherein the mRNA solution is mixed at a flow rate of about 800 ml/minute, about 1000 ml/minute, or about 12000 ml/minute; and/or

(ii) the lipid solution is mixed at a flow rate ranging from about 25-75 ml/minute, about 75-200 ml/minute, about 200-350 ml/minute, about 350-500 ml/minute, about 500-650 ml/minute, about 650-850 ml/minute, or about 850-1000 ml/minute, optionally wherein the lipid solution is mixed at a flow rate of about 100 ml/minute, about 150 ml/minute, about 200 ml/minute, about 250 ml/minute, about 300 ml/minute, about 350 ml/minute;

optionally wherein the flow rate of the mRNA solution is 2 times, 4 times, or 6 times greater than the flow rate of the lipid solution.

17. The process of any of claims 1-16, wherein the mRNA-LNP encapsulation efficiency is at least 10% higher compared to an mRNA-LNP formed from the mRNA solution mixed with the lipid solution under the same conditions except with the mRNA solution having 10 mM citrate.

18. A composition comprising mRNA encapsulated in lipid nanoparticles prepared by the process of any one of the preceding claims.

19. The composition of claim 18, wherein the composition comprises 5g or more, 10 g or more, 20 g or more, 50 g or more, 100 g or more, or 1 kg or more of mRNA.

20. The composition of claim 18 or 19, wherein:

(i) the mRNA comprises one or more modified nucleotides or wherein the mRNA is unmodified; and/or

(ii) the mRNA is greater than about 0.5 kb, 1 kb, 2 kb, 3 kb, 4 kb, 5 kb, 8 kb, 10 kb, 20 kb, 30 kb or 40 kb.