

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;

~~3. The process of claim 2, 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)~~

~~4. The process of claim 2 or 3, wherein the ambient temperature ranges from about 18-32 °C, about 21-26 °C, or about 23-25 °C.~~

~~35.~~ 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:~~

~~6. The process of any one of preceding claims, wherein(ii) the mRNA solution comprises about 3.0 mM of citrate,~~

~~7. The process of any one of claims 1-5, wherein the mRNA solution comprises about 2.5 mM of citrate,~~

~~8. The process of any one of claims 1-5, wherein the mRNA solution comprises about 2.0 mM of citrate.~~

~~9. The process of any one of claims 1-5, wherein the mRNA solution comprises 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~~

~~10. The process of any one of claims 1-5, wherein the mRNA solution comprises 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.

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

(i) the mRNA solution further comprises trehalose; and/or-

~~12. The process of any one of preceding claims, wherein(ii) the process does not require a step of heating the mRNA solution and the lipid solution prior to the mixing step.~~

813. 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:-

~~14. The process of claim 13, wherein the mRNA solution comprises about 1 g of mRNA per 8 L of the mRNA solution.~~

~~15. The process of claim 13, wherein the mRNA solution comprises about 1 g of mRNA per 4 L of the mRNA solution.~~

~~16. The process of claim 13, wherein the mRNA solution comprises or about 1 g of mRNA per 2 L of the mRNA solution; or.~~

~~17. The process of claim 13, wherein 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.~~

~~918.~~ 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:

~~19. The process of claim 18, 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.~~

~~1020.~~ The process of any one of the preceding claims, wherein:

~~(i) the mRNA solution has a pH between 3.0 and 5.0.~~

~~21. The process of claim 20, optionally wherein the mRNA solution has a pH of about 3.5, 4.0, or 4.5; and/or.~~

~~(ii)~~

~~22. The process of any one of preceding claims, wherein the mRNA solution comprises about 37.5 mM to 300 mM NaCl.~~

~~23. The process of claim 22, optionally wherein the mRNA solution comprises about 37.5 mM, about 75 mM, about 100 mM, about 150 mM, or about 300 mM NaCl.~~

~~1124.~~ 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.

~~1225.~~ The process of any one of the preceding claims, wherein the process further comprises a step of incubating the mRNA-LNPs:-

~~26.~~—The process of claim 25, optionally wherein:

(i) -the mRNA-LNPs are incubated at a temperature of between 21 °C and 65 °C, optionally:-

~~27.~~—The process of claim 26, wherein the mRNA-LNPs are incubated at a temperature of about 26 °C, about 30 °C, or about 65 °C; and/or:-

~~28.~~—The process of any one of claims 25-27, (ii) ~~wherein~~ 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:-

~~29.~~—The process of claim 28, wherein the mRNA-LNPs are incubated for about 60 minutes.

~~1330.~~ 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;:-

~~31.~~—The process of any one of the preceding claims, wherein (ii) ~~the~~ lipid solution further comprises one or more cholesterol-based lipids;:-

~~32.~~—The process of any one of preceding claims, wherein (iii) ~~the~~ mRNA-LNPs are purified by Tangential Flow Filtration;:-

(iv) ~~33.~~—The process of any one of preceding claims, wherein 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:-

~~34.~~—The process of claim 33, optionally wherein the mRNA-LNPs have an average size ranging from 40-70 nm;:-

~~35. The process of any one of claims, wherein (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;~~

~~36. The process of any one of claims, wherein the encapsulation efficiency of the mRNA-LNPs is greater than about 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%.~~

~~(vi) _____~~

~~37. The process of any one of preceding claims, wherein the mRNA-LNPs have a N/P ratio of between 1 to 10.~~

~~38. The process of claim 37, optionally wherein the mRNA-LNPs have a N/P ratio of between 2 to 6.~~

~~39. The process of claim 38, 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%;~~

~~1540. 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.~~

~~41. The process of any one of preceding claims, wherein the mRNA solution and the lipid solution are mixed by a pulse less flow pump.~~

~~42. The process of claim 41, wherein the pump is a gear pump.~~

~~43. The process of claim 42, wherein the pump is a centrifugal pump.~~

1644. 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;

~~45.~~ ~~The process of claim 44, 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;

~~46.~~ ~~The process of any one of preceding claims, wherein~~ (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;

~~47.~~ ~~The process of claim 46, 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;

~~48.~~ ~~The process of any one of claims 44-47, 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.

1849. A composition comprising mRNA encapsulated in lipid nanoparticles prepared by the process of any one of the preceding claims.

1950. The composition of claim 1849, 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.

~~5120.~~ The composition of claim ~~49-18~~ or ~~5019~~, wherein:

(i) the mRNA comprises one or more modified nucleotides or;

~~52. The composition of claim 49 or 50, wherein the mRNA is unmodified; and/or:~~

~~53. The composition of any one of claims 49-52, wherein(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.~~

~~54. The process of any of claims 1-48, 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.~~

~~55. 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 between 0.1 mM and 5 mM citrate, and wherein the mRNA-LNPs have an encapsulation efficiency of greater than 60%.~~

~~56. The process of claim 55, wherein the mRNA solution comprises between about 1 mM and 4 mM citrate.~~

~~57. The process of claim 56, wherein the mRNA solution comprises between about 2 mM and 3 mM citrate.~~

~~58. The process of claim 57, wherein the mRNA solution comprises about 2 mM citrate.~~

~~59. The process of claim 57, wherein the mRNA solution comprises about 3 mM citrate.~~