

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, and wherein the step of mixing the mRNA solution and the lipid solution comprises mixing in the presence of an amphiphilic polymer to form mRNA encapsulated within LNPs (mRNA-LNPs) in a LNP formulation solution.
2. The process of claim 1, wherein the amphiphilic polymer comprises pluronics, polyvinyl pyrrolidone, polyvinyl alcohol, polyethylene glycol (PEG), or combinations thereof, optionally wherein PEG is selected from triethylene glycol monomethyl ether (mTEG), methoxy polyethylene glycol mPEG, tetraethylene glycol monomethyl ether, pentaethylene glycol monomethyl ether, or combinations thereof, optionally wherein the PEG is triethylene glycol monomethyl ether (mTEG).
3. The process of claim 1, wherein the step of mixing the mRNA solution and the lipid solution
 - (i) yields PEG at a concentration of greater than 25% volume/volume; or
 - (ii) comprises PEG at a concentration of about 50% volume/volume.
4. The process of any one of the preceding claims, wherein the mRNA solution comprises
 - (i) less than 5 mM of citrate; and/or
 - (ii) trehalose, andwherein the mRNA-LNPs have an encapsulation efficiency of greater than 60%.
5. The process of any one of the preceding claims, wherein the mRNA solution and/or the lipid solution are at a temperature of 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,

optionally the temperature ranges from about 18-32 °C, about 21-26 °C, or about 23-25 °C, optionally wherein the process does not require a step of heating the mRNA solution and the lipid solution prior to the mixing step.

6. The process of any one of the preceding claims wherein the one or more non-cationic lipids is selected from distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoylphosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE), dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-l-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), phosphatidylserine, sphingolipids, cerebrosides, gangliosides, 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, l-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE), or a mixture thereof, optionally wherein the one or more non-cationic lipids is distearoylphosphatidylcholine (DSPC).

7. The process of any one of 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:

- (i) about 1 g of mRNA per 8 L of the mRNA solution; or
- (ii) about 1 g of mRNA per 4 L of the mRNA solution; or
- (iii) about 1 g of mRNA per 2 L of the mRNA solution; or
- (iv) a 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.

8. The process of any one of 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 at about 4:1.

9. The process of any one of preceding claims, wherein the mRNA solution has a pH between 3.0 and 5.0, optionally about 3.5, 4.0, or 4.5.
10. The process of any one of the preceding claims, wherein the step of mixing occurs in a total volume of between about 3 and 10 mL, optionally in a total volume of about 3 mL.
11. The process of any one of preceding claims, wherein the process further comprises a step of incubating the mRNA-LNPs, optionally at a temperature of between 21 °C and 65 °C, optionally about 26 °C, about 30 °C, or about 65 °C, optionally 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 about 60 minutes.
12. The process of any one of preceding claims, wherein the lipid solution does not comprise an alcohol, optionally wherein the process does not comprise an alcohol
13. The process of any one of the preceding claims, wherein the lipid solution further comprises one or more cholesterol-based lipids.
14. The process of any one of the preceding claims, wherein
- (i) the mRNA solution and the lipid solution are mixed by a pulse-less flow pump, wherein the pump is a gear pump, optionally a centrifugal pump; and/or
 - (ii) 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 about 800 ml/minute, about 1000 ml/minute, or about 12000 ml/minute; and/or
 - (iii) 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 about 100

ml/minute, about 150 ml/minute, about 200 ml/minute, about 250 ml/minute, about 300 ml/minute, about 350 ml/minute; and/or

(iv) 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; and/or

(v) the mRNA-LNPs are purified by Tangential Flow Filtration.

15. The process of any one of the preceding claims, wherein

(i) 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 an average size ranging from 40-70 nm; and/or

(ii) 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; and/or

(iii) the encapsulation efficiency of the mRNA-LNPs is greater than about 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%; and/or

(iv) wherein the mRNA-LNPs have a N/P ratio of between 1 to 10, optionally between 2 to 6, optionally about 4.

16. 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.

17. The process of any one of the preceding claims, wherein the mRNA is purified in a process free of volatile organic compounds, optionally the mRNA is purified in a process free of alcohol.

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, optionally 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.