

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, wherein the amphiphilic polymer comprises triethylene glycol monomethyl ether, methoxy polyethylene glycol, tetraethylene glycol monomethyl ether, pentaethylene glycol monomethyl ether, or combinations thereof, and wherein the lipid solution does not comprise ethanol, optionally wherein the process does not comprise ethanol.
2. The process of claim 1, wherein the amphiphilic polymer comprises triethylene glycol monomethyl ether (mTEG).
3. The process of claim 1 or 2, wherein the step of mixing the mRNA solution and the lipid solution
 - (i) yields mTEG at a concentration of 55-56% volume/volume; or
 - (ii) comprises mTEG at a concentration of 50% volume/volume.
4. The process of any one of the preceding claims, wherein the mRNA solution comprises
 - (i) citrate in the range of 0 to 5 mM, 5mM being excluded; and/or
 - (ii) trehalose, and

wherein 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 the temperature ranges from 18-32 °C, 21-26 °C, or 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-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidylethanolamine (DSPE), phosphatidylserine, sphingolipids, cerebrosides, gangliosides, 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-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:
 - (i) 1 g of mRNA per 8 L of the mRNA solution; or
 - (ii) 1 g of mRNA per 4 L of the mRNA solution; or
 - (iii) 1 g of mRNA per 2 L of the mRNA solution; or
 - (iv) a concentration of mRNA in the mRNA solution of between 0.05 mg/mL and 0.5 mg/mL, or of between 0.1 mg/mL to 0.5 mg/mL, for example of 0.1 mg/mL or of 0.35 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 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 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 3 and 10 mL, optionally in a total volume of 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 26 °C, 30 °C, or 65 °C, optionally wherein the mRNA-LNPs are incubated between 40 and 180minutes.
12. The process of any one of the preceding claims, wherein the lipid solution further comprises one or more cholesterol-based lipids.
13. 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 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 800 ml/minute, 1000 ml/minute, or 12000 ml/minute; and/or

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

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

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

(ii) the lipid nanoparticles have a PDI in the range of 0.3 to 0.1, 0.3 being excluded; and/or

(iii) the encapsulation efficiency of the mRNA-LNPs is greater than 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 4.

15. The process of any one of the preceding claims, wherein 5 g to 10 kg of mRNA is encapsulated in lipid nanoparticles in a single batch.

16. 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.

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

18. The composition of claim 17, wherein the composition comprises from 1 g to 1 kg of mRNA.

19. The composition of claim 17 or 18, wherein

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

(ii) the mRNA is in the range of 0.5 kb to 50 kb, 0.5 kb being excluded.