

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..
3. The process of claim 2, 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.
4. The process of claim 3, wherein the PEG is triethylene glycol monomethyl ether (mTEG).
5. The process of claim 1, wherein the step of mixing the mRNA solution and the lipid solution yields PEG at a concentration of greater than 25% volume/volume.
6. The process of claim 1, wherein the step of mixing the mRNA solution and the lipid solution comprises PEG at a concentration of about 50% volume/volume.
7. The process of any one of the preceding claims, wherein the mRNA solution comprises less than 5 mM of citrate, and wherein the mRNA-LNPs have an encapsulation efficiency of greater than 60%.

8. The process of any one of claims 1-7, wherein the mRNA solution and/or the lipid solution are at about ambient temperature.

9. The process of claim 8, wherein 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.

10. The process of claim 9, wherein the ambient temperature ranges from about 18-32 °C, about 21-26 °C, or about 23-25 °C.

11. 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), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoyl-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. []

12. The process of any one of claims 1-11, wherein the one or more non-cationic lipids is distearoylphosphatidylcholine (DSPC).

13. The process of any one of preceding claims, wherein the mRNA solution further comprises trehalose.

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

15. 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.
16. The process of claim 15, wherein the mRNA solution comprises about 1 g of mRNA per 8 L of the mRNA solution.
17. The process of claim 15, wherein the mRNA solution comprises about 1 g of mRNA per 4 L of the mRNA solution.
18. The process of claim 15, wherein the mRNA solution comprises about 1 g of mRNA per 2 L of the mRNA solution.
19. The process of claim 15, 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.
20. 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.
21. The process of claim 20, wherein the mRNA solution and the lipid solution are mixed at a ratio (v/v) of about 4:1.
22. The process of any one of preceding claims, wherein the mRNA solution has a pH between 3.0 and 5.0.
23. The process of claim 22, wherein the mRNA solution has a pH of about 3.5, 4.0, or 4.5.

24. 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.
25. The process of claim 24, wherein the step of mixing occurs in a total volume of about 3 mL.
26. The process of any one of the preceding claims does not comprise an alcohol.
27. The process of any one of preceding claims, wherein the process further comprises a step of incubating the mRNA-LNPs.
28. The process of claim 27, wherein the mRNA-LNPs are incubated at a temperature of between 21 °C and 65 °C.
29. The process of claim 28, wherein the mRNA-LNPs are incubated at a temperature of about 26 °C, about 30 °C, or about 65 °C.
30. The process of any one of claims 27-29, 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.
31. The process of claim 30, wherein the mRNA-LNPs are incubated for about 60 minutes.
32. The process of any one of preceding claims, wherein the lipid solution does not comprise an alcohol.

33. The process of any one of the preceding claims, wherein the lipid solution further comprises one or more cholesterol-based lipids.
34. The process of any one of preceding claims, wherein the mRNA-LNPs are purified by Tangential Flow Filtration.
35. 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.
36. The process of claim 35, wherein the mRNA-LNPs have an average size ranging from 40-70 nm.
37. The process of any one of claims, wherein 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.
38. 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%.
39. The process of any one of preceding claims, wherein the mRNA-LNPs have a N/P ratio of between 1 to 10.
40. The process of claim 39, wherein the mRNA-LNPs have a N/P ratio of between 2 to 6.
41. The process of claim 40, wherein the mRNA-LNPs have a N/P ratio of about 4.

42. The process of any one of 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.
43. The process of any one of preceding claims, wherein the mRNA solution and the lipid solution are mixed by a pulse-less flow pump.
44. The process of claim 43, wherein the pump is a gear pump.
45. The process of claim 44, wherein the pump is a centrifugal pump.
46. The process of any one of preceding claims, wherein 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.
47. The process of claim 46, wherein the mRNA solution is mixed at a flow rate of about 800 ml/minute, about 1000 ml/minute, or about 12000 ml/minute.
48. The process of any one of preceding claims, wherein 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.
49. The process of claim 48, 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.

50. The process of any one of claims 46-49, 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.
51. The process of any one of the preceding claims, wherein the mRNA is purified in a process free of volatile organic compounds.
52. The process of claim 51, wherein the mRNA is purified in a process free of alcohol.
53. A composition comprising mRNA encapsulated in lipid nanoparticles prepared by the process of any one of the preceding claims.
54. The composition of claim 51, 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.
55. The composition of claim 51 or 52, wherein the mRNA comprises one or more modified nucleotides.
56. The composition of claim 51 or 52, wherein the mRNA is unmodified.
57. The composition of any one of claims 51-54, wherein 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.