CLAIMS

1. A liquid lipid nanoparticle (LNP) formulation encapsulating mRNA encoding a peptide or polypeptide, that is resistant to aggregation and to mRNA degradation, the LNP formulation comprising:
   a. one or more LNPs having a lipid component comprising or consisting of a cationic lipid, a non-cationic lipid, a PEG-modified lipid and optionally cholesterol;
   b. mRNA encapsulated within the one or more lipid nanoparticles and encoding a peptide or polypeptide;
   c. a sugar or a sugar alcohol;
   d. a pH of from 6.0 to 8.0;
   e. a pH buffer that at a minimum buffered ionic strength provides the pH of from 6.0 to 8.0;
   f. optionally one or more additional agents that provide ionic strength to the LNP formulation;

   wherein a total concentration of pH buffer from (e.), and optionally one or more additional agents from (f.), provide(s) an ionic strength of the LNP formulation that is at least two times greater than the minimum buffered ionic strength.

2. The LNP formulation of claim 1, wherein following three rounds of freezing at -20°C and rethawing, the LNP formulation exhibits (i) less aggregation, (ii) less degradation of the encapsulated mRNA, or (iii) both (i) and (ii), as compared to an identical LNP formulation that has only the minimum buffered ionic strength in the LNP formulation instead of an ionic strength that is at least two times greater than the minimum buffered ionic strength.

3. The LNP formulation of claim 1, wherein the non-cationic lipid is selected from 1,2-Dierucoyl-sn-glycero-3-phosphoethanolamine (DEPE), 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-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, or 1-stearoyl-2-oleoyl-phosphatidyethanolamine (SOPE).

4. The LNP formulation of claim 3, wherein the non-cationic lipid is dioleoylphosphatidylethanolamine (DOPE).
5. The LNP formulation of claim 4, wherein the DOPE is at a lipid molar ratio of 10% or greater.
6. The LNP formulation of any one of the preceding claims, wherein the cationic lipid is a lipidoid, optionally wherein the lipidoid is a lipid molar ratio of 40%-60%.
7. The LNP formulation of any one of the preceding claims, wherein the mRNA encodes a protein deficient in a subject or a vaccine antigen.

8. The LNP formulation of any one of the preceding claims, wherein the sugar or sugar alcohol is selected from the group consisting of dextrose, sorbitol, trehalose, sucrose, raffinose, dextran, and inulin.
9. The LNP formulation of any one of the preceding claims, wherein the sugar is a disaccharide at a concentration of about 1% - 20%.

10. The LNP formulation of claim 9, wherein the disaccharide is at a concentration of about 2.5-3.0%.
11. The LNP formulation of claim 10, wherein the disaccharide to the pH buffer ratio is between 0.2 – 0.5.
12. The LNP formulation of any one of claims 9-10, wherein the disaccharide is trehalose.
13. The LNP formulation of any one of the preceding claims, wherein the pH is between about 6.0 and about 8.0.
14. The LNP formulation of claim 1, wherein the pH is 7.4.

15. The LNP formulation of any one of the preceding claims, wherein the pH buffer has a pKa between 6.0 and 8.2.
16. The LNP formulation of claim 15, wherein the buffer is selected from the group consisting of a phosphate buffer, a citrate buffer, an imidazole buffer, a histidine buffer, and a Good's buffer.
17. The LPN formulation of claim 16, wherein the Good's buffer is a Tris buffer or HEPES buffer.
18. The LPN formulation of claim 16or 17, wherein the pH buffer is a phosphate buffer (*e.g.*, a citrate-phosphate buffer), a Tris buffer, or an imidazole buffer.
19. The LNP formulation of any one of the preceding claims, wherein the minimum buffered ionic strength is at least 75 mM.
20. The LNP formulation of claim 19, wherein the minimum buffered ionic strength is about 75 mM – 200 mM.
21. The LNP formulation of claim 20, wherein the minimum buffered ionic strength is between 100 mM – 200 mM.
22. The LNP formulation of any one of the preceding claims, wherein the one or more agents that provide ionic strength comprises a salt or a sugar.
23. The LNP formulation of claim 22, wherein the salt is selected from the group consisting of NaCl, KCl, and $CaCl_2$, and wherein the sugar is trehalose.
24. The LNP formulation of any one of claims 1-20, wherein the total concentration of the one or more additional agents that provide ionic strength is between about 50-300.
25. The LNP formulation of claim 24, wherein the total concentration of pH buffer is between about 15-250 mM.
26. The LNP formulation of claim 24, wherein the total concentration of the pH buffer and the one or more additional agents that provide ionic strength is selected from about 40 mM Tris buffer and about 50-200 mM NaCl, about 50 mM Tris buffer and about 50 mM – 200 mM NaCl, about 100 mM Tris buffer and about 50 mM – 200 mM NaCl, about 40 mM imidazole and about 50 mM – 200 mM NaCl, about 50 mM imidazole and 50 mM-200 mM NaCl, about 100 mM imidazole and 50 mM – 200 mM NaCl, about 40 mM phosphate and 50 mM – 200 mM NaCl, about 50 mM phosphate and 50 – 200 mM NaCl, about 100 mM phosphate and 50 – 200 mM NaCl.

27. The LNP formulation of any one of the preceding claims, wherein the ionic strength of the LNP formulation is at least 2.25 times greater than, at least 2.5 times greater than, at least 2.75 times greater than, at least 3 times greater than, at least 3.5 times greater than, at least 4 times greater than, at least 4.5 times greater than, at least 5 times greater than, the minimum buffered ionic strength.
28. The LNP formulation of any one of claims 1-26, wherein the ionic strength of the LNP formulation is at least two times greater and less than 5 times the minimum buffered ionic strength.
29. The LNP formulation of any one of the preceding claims, wherein the ionic strength of the LNP formulation is at least two times greater and less than 20 times greater than the minimum buffered ionic strength, and wherein the ionic strength of the LNP formulation is between about 150 mM -750 mM.
30. The LNP formulation of any one of the preceding claims, wherein the ionic strength of the LNP formulation is at least two times greater and less than 20 times greater than the minimum buffered ionic strength, and wherein the ionic strength of the LNP formulation is greater than 150 mM.

31. The LNP formulation of any one of the preceding claims, wherein following more than three rounds of freezing at -20°C and rethawing, the LNP formulation exhibits (i) less aggregation, (ii) less degradation of the encapsulated mRNA, or (iii) both (i) and (ii), as compared to an identical LNP formulation that has only the minimum buffered ionic strength in the LNP formulation instead of an ionic strength that is at least two times greater than the minimum buffered ionic strength.
32. The LNP formulation of any one of the preceding claims, wherein the LNPs have a diameter of less than about 100 nm.
33. The LNP formulation of claim 30, wherein the LNPs have a diameter between about 70 nm – 90 nm.
34. The LNP formulation of any one of the preceding claims, wherein the lipid component comprises or consists of DMG-PEG-2000, cKK-E10, cholesterol, and DOPE.

35. The LNP formulation of any one of the preceding claims, wherein the N/P ratio is between about 3-5.

36. The LNP formulation of any one of the preceding claims, wherein the mRNA is at a final concentration of between about 0.05 mg/mL and 1.0 mg/mL.

37. The LNP formulation of claim 36, wherein the mRNA is at a concentration of between about 0.2 mg/mL and 0.5 mg/mL.

38. The LNP formulation of any one of the preceding claims, wherein the LNPs are stable at -20°C for at least 3 months.

39. The LNP formulation of any one of the preceding claims, wherein subcutaneous or intramuscular delivery of the formulation is accompanied with reduced pain in comparison to a formulation that does not comprise a buffer having a concentration of or below 300 mM and a pH of between about 7.0 and 7.5, wherein the reduced pain is assessed by a 10-cm visual analog scale (VAS) or a six-item verbal rating scale (VRS).

40. A method of reducing LNP degradation and/or aggregation, the method comprising storing the LNP in the LNP formulation of any one of the preceding claims.

41. The LNP formulation of claim 1, wherein:

the sugar is a disaccharide;

the disaccharide to the pH buffer ratio is between 0.2 – 0.5;

the one or more additional agents that provide ionic strength to the LNP formulation comprise a salt at a concentration of 50-150 mM;

the minimum buffered ionic strength is between 75 mM to 200 mM;

the ionic strength of the LNP formulation is at least two times greater and less than 20 times greater than the minimum buffered ionic strength, wherein the ionic strength of the LNP formulation is between about 150 mM - 750 mM; and

the total concentration of the pH buffer is between 100-300 mM if the LNP formulation does not comprise the optional one or more agents that provide ionic strength

or the pH buffer is between 40-50 mM if the LNP formulation comprises the salt at the concentration of 50-300 mM .

42. The LNP formulation of claim 41, wherein the disaccharide is trehalose, the salt is selected from the group consisting of NaCl, KCl, and $CaCl_2$, and the ionic strength of the LNP formulation is between about 150 mM - 400 mM.

43. The LNP formulation of claim 41or 42, wherein the pH is between 6.0 and 7.0.