

CLAIMS

1. A liquid lipid nanoparticle (LNP) formulation that is resistant to aggregation and to mRNA degradation, the LNP formulation comprising:
 - a. one or more lipid nanoparticles (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 LNPs and encoding a peptide or polypeptide, wherein the mRNA is at a final concentration of between 0.05 mg/mL and 1.0 mg/mL;
 - c. a pH buffer having a concentration between 30-150 mM or a pH buffer having a concentration between 100-300 mM;
 - d. a disaccharide, wherein the disaccharide to pH buffer ratio is between 0.1 – 0.9; and
 - e. a salt that provides increased ionic strength in addition to the ionic strength of the pH buffer to the LNP formulation, wherein:

the LNP formulation has a total ionic strength between 150 mM – 750 mM and a pH between 6.0 and 8.0; and

the total ionic strength of the LNP formulation between 150 mM – 750 mM is at least two times greater than the minimum buffered ionic strength of an otherwise identical LNP formulation that has only a minimum buffered ionic strength for providing the same pH as the LNP formulation.
2. 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), palmitoyl-oleoylphosphatidylcholine (POPC), palmitoyl-oleoylphosphatidylethanolamine (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-phosphatidylethanolamine (SOPE).

3. The LNP formulation of claim 2, wherein the non-cationic lipid is dioleoylphosphatidylethanolamine (DOPE).
4. The LNP formulation of claim 3, wherein the DOPE is at a lipid molar ratio of 10% or greater.
5. The LNP formulation of any one of the preceding claims, wherein the cationic lipid is a lipidoid, optionally wherein the lipidoid is at a lipid molar ratio of 40%-60%.
6. The LNP formulation of any one of claims 1-5, wherein the mRNA encodes a protein deficient in a subject.
7. The LNP formulation of any one of claims 1-5, wherein the mRNA encodes a vaccine antigen.
8. The LNP formulation of any one of the preceding claims, wherein the disaccharide is trehalose or sucrose.
9. The LNP formulation of any one of the preceding claims, wherein the disaccharide to pH buffer ratio is between 0.2 – 0.5.
10. The LNP formulation of any one of the preceding claims, wherein the pH buffer comprises a phosphate buffer, a citrate buffer, an imidazole buffer, a histidine buffer, and a Good's buffer.
11. The LNP formulation of any one of the preceding claims, wherein the LNPs have a diameter less than 100 nm, optionally between 70 nm – 90 nm, and wherein the N/P ratio is between 3:1 and 5:1.

12. 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.
13. The LNP formulation of any one of the preceding claims, wherein the salt is NaCl, KCl, or CaCl₂.
14. The LNP formulation of any one of claims 1-13, wherein the total ionic strength of the LNP formulation is between 150 mM – 400 mM.
15. The LNP formulation of any one of claims 1-13, wherein the pH buffer and the salt are selected from:
 - 40 mM Tris buffer and 75-200 mM NaCl;
 - 50 mM Tris buffer and 75 mM – 200 mM NaCl;
 - 100 mM Tris buffer and 75 mM – 200 mM NaCl;
 - 40 mM imidazole buffer and 75 mM – 200 mM NaCl;
 - 50 mM imidazole buffer and 75 mM-200 mM NaCl;
 - 100 mM imidazole buffer and 75 mM – 200 mM NaCl;
 - 40 mM phosphate buffer and 75 mM – 200 mM NaCl;
 - 50 mM phosphate buffer and 75 – 200 mM NaCl;
 - 100 mM phosphate and 75 – 200 mM NaCl.