CLAIMS

We claim:

1. A stable composition comprising lipid nanoparticles encapsulating messenger RNA mRNA, wherein the mRNA encodes a protein or a peptide, wherein each of the lipid nanoparticles comprises one or more cationic lipids, one or more non-cationic lipids and less than 0.5% of PEG-modified lipids or PEG, and wherein the lipid nanoparticles encapsulating the mRNA are stable following one or more freeze thaws.

2. The stable composition of claim 1, wherein each of the lipid nanoparticles comprises one cationic lipid, dioleoylphosphatidylethanolamine (DOPE), and less than about 0.5% of PEG-modified lipids or PEG.

3. The stable composition of claim 1 or 2, wherein the lipid nanoparticles encapsulating the mRNA maintain an average diameter within 50% of the original average size following one or more freeze thaw cycles.

4. The stable composition of any one of the preceding claims, wherein the lipid nanoparticles encapsulating the mRNA maintains an average diameter within 10% of the original average size following one or more freeze thaw cycles.

5. The stable composition of any one of the preceding claims, wherein the lipid nanoparticles encapsulating the mRNA maintains an average diameter within 5% of the original average size following one or more freeze thaw cycles.

6. The stable composition of any one of the preceding claims, wherein the lipid nanoparticles have an mRNA encapsulation efficiency of between about 50% and 99%.

7. The stable composition of any one of the preceding claims, wherein each of the lipid nanoparticles further comprises a cholesterol-based lipid.

8. The stable composition of any one of the preceding claims, wherein each of the lipid nanoparticles comprises 0.4% of PEG-modified lipids or less, 0.3% of PEG-modified lipids or less, 0.2% of PEG-modified lipids or less, or 0.1% of PEG-modified lipids or less.

9. The stable composition of any one of the preceding claims, wherein each of the lipid nanoparticles is substantially free of PEG-modified lipids.

10. The stable composition of any one of the preceding claims, wherein each of the lipid nanoparticles comprises an amphiphilic block copolymer.

11. The stable composition of claim 10, wherein each of the lipid nanoparticles comprises less than 3% amphiphilic block copolymer, less than 2.5% amphiphilic block copolymer, less than 2% amphiphilic block copolymer, less than 1.5% amphiphilic block copolymer, less than 1% amphiphilic block copolymer, less than 0.5% amphiphilic block copolymer, less than 0.05% amphiphilic block copolymer, or less than 0.01% amphiphilic block copolymer.

12. The stable composition of claim 11, wherein the composition comprises less than 0.05 %, less than 0.04%, less than 0.03%, less than 0.02%, or less than 0.01% amphiphilic block copolymer of the total composition by weight.

13. The stable composition of claim 12, wherein the composition comprises a residual of amphiphilic block copolymer.

14. The stable composition of any one of claims 10-13, wherein the amphiphilic block copolymer is a poloxamer.

15. The stable composition of claim 14, wherein the poloxamer is selected from poloxamer 84, poloxamer 101, poloxamer 105, poloxamer 108, poloxamer 122, poloxamer 123, poloxamer 124, poloxamer 181, poloxamer 182, poloxamer 183, poloxamer 184, poloxamer 185, poloxamer 188, poloxamer 212, poloxamer 215, poloxamer 217,

poloxamer 231, poloxamer 234, poloxamer 235, poloxamer 237, poloxamer 238, poloxamer 282, poloxamer 284, poloxamer 288, poloxamer 304, poloxamer 331, poloxamer 333, poloxamer 334, poloxamer 335, poloxamer 338, poloxamer 401, poloxamer 402, poloxamer 403, poloxamer 407 or combination thereof.

16. A stable composition comprising lipid nanoparticles encapsulating a messenger RNA (mRNA) that encodes a protein or a peptide, wherein each of the lipid nanoparticles comprises one or more cationic lipids, one or more non-cationic lipids, a poloxamer and is substantially free of PEG-modified lipids or PEG, and wherein the lipid nanoparticles encapsulating the mRNA are stable following one or more freeze thaw cycles.

17. A stable composition comprising lipid nanoparticles encapsulating a messenger RNA (mRNA) that encodes a protein or a peptide, wherein each of the lipid nanoparticles comprises one or more cationic lipids, one or more non-cationic lipids, a poloxamer and is substantially free of PEG-modified lipids or PEG, and wherein the lipid nanoparticles encapsulating the mRNA generate low to no anti-PEG antibodies, and/or reduce accelerated blood clearance (ABC).

18. The stable composition of claim 17, wherein the poloxamer is present in the lipid nanoparticles at an amount of less than 0.1%.

19. The stable composition of any one of claims 16-18, wherein the poloxamer is present in the lipid nanoparticles at an amount of at less than 0.05%.

20. The stable composition of any one of claims 16-19, wherein the non-cationic lipid is dioleoylphosphatidylethanolamine (DOPE).

21. The stable composition of any one of claims 16-20, wherein the lipid nanoparticle maintain an average diameter within 50% of the original average size following one or more freeze thaw cycles.

22. The stable composition of claim 21, wherein the lipid nanoparticles maintain an average diameter within 10% of the original average size following one or more freeze thaw cycles.

23. The stable composition of claim 22, wherein the lipid nanoparticles maintain an average diameter within 5% of the original average size following one or more freeze thaw cycles.

24. The stable composition of any one of claims 16-23, wherein the lipid nanoparticles have an mRNA encapsulation efficiency of between about 50% and 99%.

25. The stable composition of any one of claims 16-24, wherein each of the lipid nanoparticles further comprises cholesterol-based lipid.

26. The stable composition of any one of claims 16-25, wherein each of the lipid nanoparticles does not comprise cholesterol-based lipid.

27. The stable composition of any of claims 16-26, wherein each of the lipid nanoparticles is a two-component lipid nanoparticle.

28. The stable composition of any one of claims 16-27, wherein the poloxamer has ethylene oxide units from about 10 to about 150.

29. The stable composition of claim 28, wherein the poloxamer has propylene oxide units from about 10 to about 100.

30. The stable composition of any one of claims 16-29, wherein the poloxamer has an average molecular weight of about 4,000 g/mol to about 20,000 g/mol.

31. The stable composition of any one of claims 16-30, wherein the poloxamer is selected from poloxamer 84, poloxamer 101, poloxamer 105, poloxamer 108, poloxamer 122, poloxamer 123, poloxamer 124, poloxamer 181, poloxamer 182, poloxamer 183,

poloxamer 184, poloxamer 185, poloxamer 188, poloxamer 212, poloxamer 215, poloxamer 217, poloxamer 231, poloxamer 234, poloxamer 235, poloxamer 237, poloxamer 238, poloxamer 282, poloxamer 284, poloxamer 288, poloxamer 304, poloxamer 331, poloxamer 333, poloxamer 334, poloxamer 335, poloxamer 338, poloxamer 401, poloxamer 402, poloxamer 403, poloxamer 407 or combination thereof.

32. The stable composition of any one of the preceding claims, wherein the lipid nanoparticles have an average size of less than about 200 nm.

33. The stable composition of claim 32, wherein the average size is about 150 nm, 140 nm, 130 nm, 120 nm, 110 nm, 100 nm or less.

34. The stable composition of any one of the preceding claims, wherein the lipid nanoparticles have a polydispersity index (PDI) of 0.25 or less, 0.2 or less, 0.15 or less, 0.1 or less.

35. A method for delivery of messenger RNA (mRNA) for *in vivo* production of a protein or a peptide, comprising administering to a subject a stable composition according to any one of the preceding claims.

36. A method for delivery of messenger RNA (mRNA) for *in vivo* production of a protein or a peptide, comprising administering to a subject a stable composition according to any one of the preceding claims, wherein the administering the stable composition does not results in anti-PEG antibodies and/or accelerated blood clearance (ABC) in the subject.

37. A method of treating a subject having a deficiency in a protein or peptide, comprising administering to a subject in need of treatment a stable composition according to any one of claims 1-33.

38. The method of any one of claims 35-37, wherein the stable composition is administered by intravenous injection.

39. The method of any one of claims 35-37, wherein the stable composition is administered by pulmonary delivery.

40. The method of any one of claims 35-37, wherein the stable composition is administered by intramuscular delivery.

41. The method of any one of claims 35-40, wherein the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 12, 24, 36, 48, 60, or 72 hours after administration.

42. A process of encapsulating messenger RNA (mRNA) in lipid nanoparticles comprising a step of mixing an mRNA solution and a lipid solution in the presence of a poloxamer.

43. The process of claim 42, wherein the lipid solution comprises one or more cationic lipids, one or more non-cationic lipids and less than 0.5% of PEG-modified lipids or PEG.

44. The process of claim 42 or 43, wherein the lipid solution comprises pre-formed lipid nanoparticles.

45. The process of any one of claims 42-44, wherein the mRNA solution and/or the lipid solution are at a pre-determined temperature higher than ambient temperature.

46. The process of any one of claims 42-45, wherein the poloxamer is first added to the mRNA solution.

47. The process of any one of claims 42-46, wherein the poloxamer is present in an amount lower than its critical micelle concentration (CMC).

48. The process of claim 47, wherein the poloxamer is present in an amount about 1%, about 2%, about 3%, about 4%, about 5%, about 6%, about 7%, about 8%, about 9%, about

10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, or about 50% lower than its CMC.

49. The process of claim 47, wherein the poloxamer is present in an amount less than about 50% of its CMC.

50. The process of any one of claims 42-49, wherein the process further comprises a step of removing the poloxamer.

51. The process of claim 50, wherein the poloxamer is removed by dialysis.

52. The process of claim 50 or 51, wherein less than about 0.05% poloxamer remains upon removal.

53. The process of claim 52, wherein less than about 0.01% poloxamer remains upon removal.

54. The process of claim 52, wherein a residual amount of poloxamer remains upon removal.

55. The process of any one of claims 50-54, wherein the amount of poloxamer remaining after removal is undetectable.

56. The process of any one of claims 42-55, wherein the poloxamer has ethylene oxide units from about 10 to about 150.

57. The process of claim 56, wherein the poloxamer has propylene oxide units from about 10 to about 100.

58. The process of any one of claims 42-57, wherein the poloxamer has an average molecular weight of about 4,000 g/mol to about 20,000 g/mol.

59. The process of any one of claims 42-58, wherein the poloxamer is selected from poloxamer 84, poloxamer 101, poloxamer 105, poloxamer 108, poloxamer 122,

poloxamer 123, poloxamer 124, poloxamer 181, poloxamer 182, poloxamer 183, poloxamer 184, poloxamer 185, poloxamer 188, poloxamer 212, poloxamer 215, poloxamer 217, poloxamer 231, poloxamer 234, poloxamer 235, poloxamer 237, poloxamer 238, poloxamer 282, poloxamer 284, poloxamer 288, poloxamer 304, poloxamer 331, poloxamer 333, poloxamer 334, poloxamer 335, poloxamer 338, poloxamer 401, poloxamer 402, poloxamer 403, poloxamer 407 or combination thereof.

60. The process of any one of claims 42-59, wherein the non-cationic lipid is dioleoylphosphatidylethanolamine (DOPE).

61. The process of any one of claims 42-59, wherein the process does not include mixing any cholesterol lipids.

62. The process of any one of claims 42-61, wherein the lipid nanoparticles have an encapsulation efficiency of at least 50%.

63. The process of claim 42-61, wherein the lipid nanoparticles have an encapsulation efficiency of between about 60% and 99%.

64. The process of any one of claims 42-63, wherein the lipid nanoparticles have an average size of about 200 nm or less.

65. The process of claim 64, wherein the lipid nanoparticles have an average size of about 150 nm or less, 140 nm or less, 130 nm or less, 120 nm or less, 110 nm or less, or about 100 nm or less.

66. A composition comprising lipid nanoparticles encapsulating mRNA formed according to a process of any one of claims 42-63.