CLAIMS:

1. A lipid nanoparticle ("LNP") composition comprising:

an RNA component, wherein the RNA component comprises an mRNA encoding an RNA-guided DNA- binding agent,; and

a lipid component, wherein the lipid component comprises:

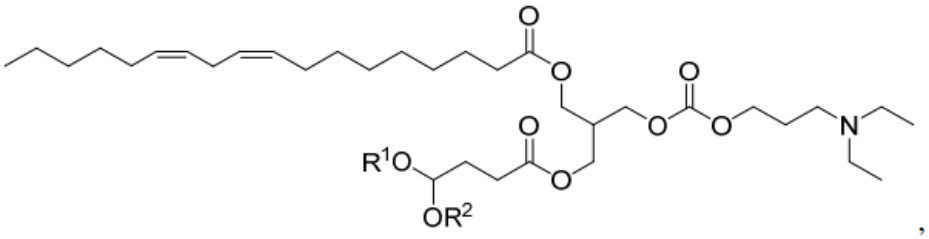
50-60 mol-% amine lipid;

8-10 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is about 6.

2. An LNP composition comprising:

an RNA component, wherein the RNA component comprises an mRNA encoding an RNA-guided DNA- binding agent,;

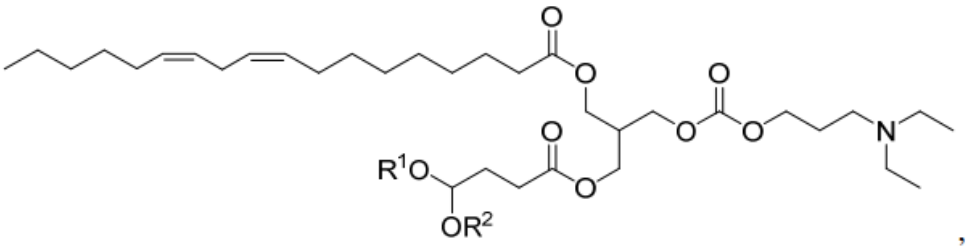
50-60 mol-% amine lipid;

27-39.5 mol-% helper lipid;

8-10 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and the N/P ratio of the LNP composition is 5-7.

3. The LNP composition of claim 2, wherein the N/P ratio is about 6.

4. The LNP composition of claim 7, wherein the lipid component comprises

50-60 mol-% amine lipid;

5-15 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid.

5. An LNP composition comprising:

an RNA component, wherein the RNA component comprises an mRNA encoding an RNA-guided DNA- binding agent, ; and

a lipid component, wherein the lipid component comprises

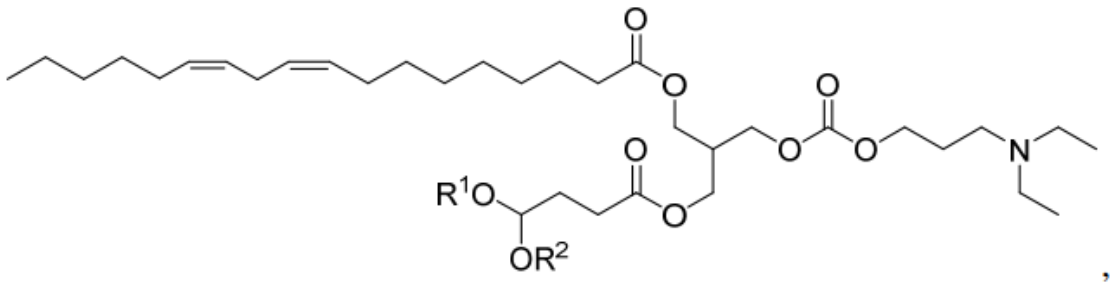
40-60 mol-% amine lipid;

5-15 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and the N/P ratio of the LNP composition is about 6.

6. An LNP composition comprising:

an RNA component, wherein the RNA component comprises an mRNA encoding an

RNA-guided DNA- binding agent, ; and

a lipid component, wherein the lipid component comprises

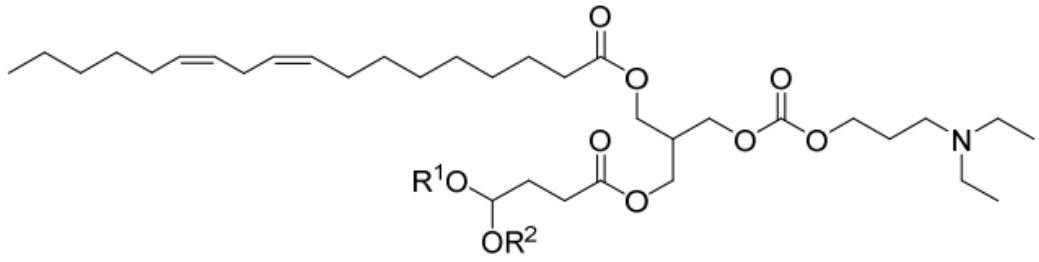
50-60 mol-% amine lipid;

5-15 mol-% neutral lipid; and

1.5-10 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is about 6.

7. A lipid nanoparticle (LNP) composition comprising:

an RNA component, wherein the RNA component comprises an mRNA encoding an RNA-guided DNA-binding agent; and

a lipid component, wherein the lipid component comprises

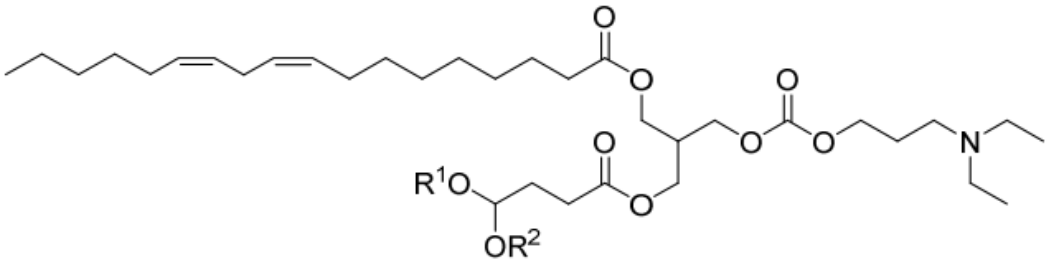
40-60 mol-% amine lipid;

0-10 mol-% neutral lipid; and

1.5-10 mol-% PEG lipid, wherein

the remainder of the lipid component is helper lipid,

the amine lipid is represented by the following structural formula



,

wherein R^1 and R^2 are each independently a C4-C12 alkyl, and

the N/P ratio of the LNP composition is 5-7.

8. The LNP composition of claim 7,
wherein the lipid component comprises:
8-10 mol-% neutral lipid;

9. The LNP composition of any one of claims 2 or 7, wherein the N/P ratio of the LNP composition is about 6.

10. The LNP composition of any one of claims 1-9, wherein the mRNA encodes a Class 2 Cas nuclease mRNA.

11. The LNP composition of any one of claims 1-10, wherein the mRNA encodes a Cas9 nuclease mRNA.

12. The LNP composition of any one of claims 1-11, wherein the mRNA is a modified mRNA.

13. The LNP composition of any one of claims 1-12, wherein the mRNA comprises an open reading frame encoding the RNA-guided DNA-binding agent, wherein the open reading frame has a uridine content from its minimum uridine content to 150% of the minimum uridine content.

14. The LNP composition of any one of claims 1-13, wherein the mRNA comprises an open reading frame encoding the RNA-guided DNA-binding agent, wherein the open reading frame has a uridine dinucleotide content from its minimum uridine dinucleotide content to 150% of the minimum uridine dinucleotide content.

15. The LNP composition of any one of claims 1-14 wherein the mRNA comprises a sequence with at least 90% identity to any one of SEQ ID NO: 1, 4, 7, 9, 10, 11, 12, 14, 15, 17, 18, 20, 21, 23, 24, 26, 27, 29, 30, 50, 52, 54, 65, or 66.

16. The LNP composition of any one of claims 1-15, wherein the RNA component further

comprises a gRNA nucleic acid.

17. The LNP composition of claim 16, wherein the gRNA nucleic acid is a gRNA.

18. The LNP composition of claim 16, wherein the gRNA nucleic acid is a dual-guide RNA (dgRNA).

19. The LNP composition of claim 16, wherein the gRNA nucleic acid a single guide RNA (sgRNA).

20. The LNP composition of claim 17, wherein the gRNA is modified.

21. The LNP composition of claim 20, wherein the gRNA comprises a modification selected from 2'-O-methyl (2'-O-Me) modified nucleotide, a phosphorothioate (PS) bond between nucleotides; and a 2'-fluoro (2'-F) modified nucleotide.

22. The LNP composition of claim 20 or 21, wherein the gRNA comprises a modification at one or more of the first five nucleotides at the 5' end.

23. The LNP composition of any one of claims 20-22, wherein the gRNA comprises a modification at one or more of the last five nucleotides at the 3' end.

24. The LNP composition of any one of claims 20-23, wherein the gRNA comprises PS bonds between the first four nucleotides at the 5' end.

25. The LNP composition of any one of claims 20-24, wherein the gRNA comprises PS bonds between the last four nucleotides at the 3' end.

26. The LNP composition of any one of claims 20-25, wherein the gRNA further comprises 2'-O-Me modified nucleotides at the first three nucleotides at the 5' end.

27. The LNP composition of any one of claims 20-26, wherein the gRNA further comprises 2'-O-Me modified nucleotides at the last three nucleotides at the 3' end.

28. The LNP composition of any one of claims 1-27, further comprising at least one template nucleic acid.

29. The LNP composition of any one of claims 1-28, wherein the lipid component comprises about 3 mol-% PEG lipid.

30. The LNP composition of any one of claims 1-29, wherein the lipid component comprises about 2.5 mol-% PEG lipid.

31. The LNP composition of any one of claims 1-30, wherein the lipid component comprises about 50 mol-% amine lipid.

32. The LNP composition of any one of claims 1-31, wherein the lipid component comprises about 55 mol-% amine lipid.

33. The LNP composition of any one of claims 1-32, wherein the lipid component comprises 47-53 mol-% ~~mol %~~ amine lipid.

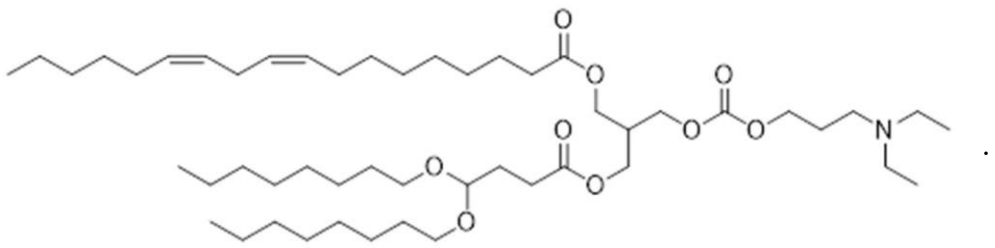
34. The LNP composition of any one of claims 1-33, wherein the lipid component comprises 48-53 mol-% amine lipid.

35. The LNP composition of any one of claims 1-36, wherein the lipid component comprises 53-57 mol-% amine lipid.

36. The LNP composition of any one of claims 1-35, wherein the N/P ratio is 6 ± 1.

37. The LNP composition of any one of claims 1-36, wherein the N/P ratio is 6 ± 0.5.

38. The LNP composition of any one of claims 1-37, wherein the amine lipid is Lipid A, wherein Lipid A is represented by the following structural formula:



39. The LNP composition of any one of claims 1-37, wherein R^1 and R^2 is each independently a C5-C12 alkyl.

40. The LNP composition of any one of claims 1-37, wherein R^1 and R^2 is each independently a C5-C10 alkyl.

41. The LNP composition of any one of claims 1-37, wherein R^1 and R^2 are each independently selected from a C4, C5, C6, C7, C8, C9, C10, C11, and C12 alkyl.

42. The LNP composition of any one of claims 1-41, wherein the helper lipid is a steroid, sterol, or alkyl resorcinol.

43. The LNP composition of any one of claims 1-41, wherein the helper lipid is selected from cholesterol, 5-heptadecylresorcinol, and cholesterol hemisuccinate.

44. The LNP composition of any one of claims 1-41, wherein the helper lipid is cholesterol.

45. The LNP composition of any one of claims 1-44, wherein the neutral lipid is a neutral

phospholipid.

46. The LNP composition of any one of claims 1-44, wherein the neutral lipid is selected from dipalmitoylphosphatidylcholine (DPPC), distearoylphosphatidylcholine (DSPC), dioleylphosphatidylcholine (DOPC), dimyristoylphosphatidylcholine (DMPC), 1-palmitoyl- 2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC), 1,2-diarachidoyl-*sn*-glycero-3- phosphatidylcholine (DAPC), phosphatidylethanolamine (PE), egg phosphatidylcholine (EPC), dilauroylphosphatidylcholine (DLPC), 1-myristoyl-2-palmitoyl phosphatidylcholine (MPPC), 1-palmitoyl-2-myristoyl phosphatidylcholine (PMPC), 1-palmitoyl-2-stearoyl phosphatidylcholine (PSPC), 1,2-dibehenoyl-*sn*-glycero-3-phosphocholine (DBPC), 1- stearoyl-2-palmitoyl phosphatidylcholine (SPPC), 1,2-dieicosenoyl-*sn*-glycero-3- phosphocholine (DEPC), palmitoyloleoyl phosphatidylcholine (POPC), lysophosphatidyl choline, dioleoyl phosphatidylethanolamine (DOPE), dilinoleoylphosphatidylcholine, distearoylphosphatidylethanolamine (DSPE), dimyristoyl phosphatidylethanolamine (DMPE), dipalmitoyl phosphatidylethanolamine (DPPE), palmitoyloleoyl phosphatidylethanolamine (POPE), lysophosphatidylethanolamine, and combinations thereof.

47. The LNP composition of any one of claims 1-44, wherein the neutral lipid is DSPC.

48. The LNP composition of any one of claims 1-44, wherein the neutral lipid is DPPC.

49. The LNP composition of any one of claims 1-44, wherein the neutral lipid is dimyristoyl phosphatidylethanolamine (DMPE).

50. The LNP composition of any one of claims 1-49, wherein the PEG lipid comprises dimyristoylglycerol (DMG).

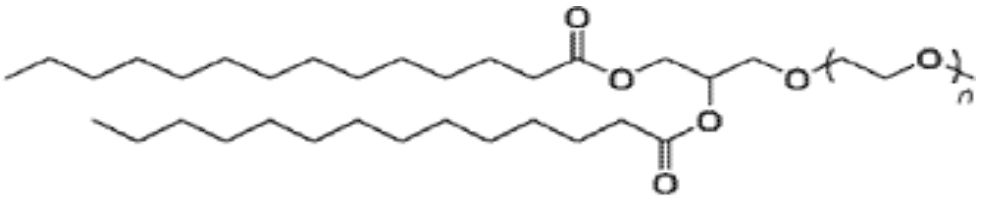
51. The LNP composition of any one of claims 1-50, wherein the PEG lipid comprises a PEG-2k.

52. The LNP composition of any one of claims 1-49, wherein the PEG lipid is selected from PEG-dilauroylglycerol, PEG-dimyristoylglycerol (PEG-DMG), PEG-dipalmitoylglycerol, PEG-distearoylglycerol (PEG-DSPE), PEG-dilaurylglycamide, PEG- dimyristylglycamide, PEG-dipalmitoylglycamide, PEG-distearoylglycamide, PEG- cholesterol (1-[8'-(Cholest-5-en-3[beta]-oxy)carboxamido-3',6'-dioxaoctanyl]carbamoyl- [omega]-methyl-poly(ethylene glycol), PEG-DMB (3,4-ditetradecoxylbenzyl-[omega]- methyl-poly(ethylene glycol)ether), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N- [methoxy(polyethylene glycol)-2000] (PEG2k-DMG), 1,2-distearoyl-*sn*-glycero-3- phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSPE), 1,2- distearoyl-*sn*-glycerol, methoxypolyethylene glycol (PEG2k-DSG), poly(ethylene glycol)- 2000-dimethacrylate (PEG2k-DMA), and 1,2-distearyloxypropyl-3-amine-N-[methoxy(polyethylene glycol)-2000] (PEG2k-DSA).

53. The LNP composition of any one of claims 1-49, wherein the PEG lipid is a PEG-DMG.

54. The LNP composition of claim 53, wherein the PEG-DMG is a PEG2k-DMG.

55. The LNP composition of any one of claims 1-49, wherein the PEG lipid has the following structural formula:



DMG-PEG ,

wherein n represents the number average degree of polymerization of ethylene oxide subunits, and n is 40-50.

56. The LNP composition of any one of claims 1-55, wherein the RNA component comprises a Class 2 Cas nuclease mRNA and a gRNA.

57. The LNP composition of claim 56, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio from 10:1 to 1:10 by weight.

58. The LNP composition of claim 56, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio from 5:1 to 1:5 by weight.

59. The LNP composition of claim 56, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio from 3:1 to 1:1 by weight.

60. The LNP composition of claim 56, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio from 2:1 to 1:1 by weight.

61. The LNP composition of claim 56, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio of about 2:1 by weight

62. The LNP composition of claim 56, wherein the gRNA and Class 2 Cas nuclease mRNA are present in a ratio of about 1:1 by weight.

63. An in vitro method of gene editing, comprising contacting a cell with at least one LNP composition of any one of claims 56-62.

64. An in vitro method of gene editing, comprising delivering a Class 2 Cas nuclease mRNA and a guide RNA nucleic acid to a cell, wherein the Class 2 Cas nuclease mRNA and the guide RNA nucleic acid are formulated as at least one LNP composition of any one of claims 56-62.

65. An in vitro method of producing a genetically engineered cell, comprising contacting a cell with at least one LNP composition of any one of claims 56-62.

66. The method of any one of claims 63-65, wherein the LNP composition is administered at least two times.

67. The method of claim 66, wherein the LNP composition is administered 2-5 times.

68. The method of claim 66 or 67, wherein editing improves upon readministration.

69. The method of any one of claims 63-68, further comprising introducing at least one template nucleic acid to the cell.

70. A lipid nanoparticle (LNP) composition comprising:

an RNA component, wherein the RNA component comprises an mRNA encoding an RNA-guided DNA-binding agent; and

a lipid component, wherein the lipid component comprises:

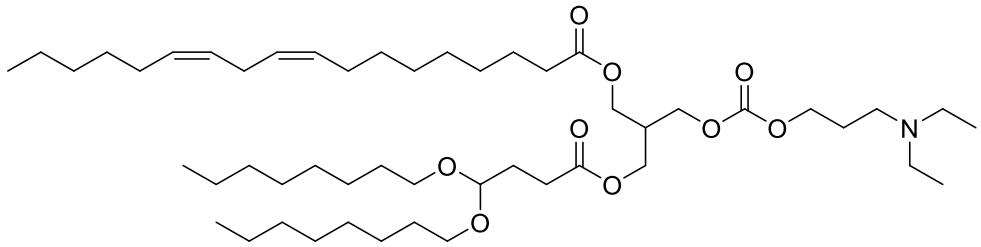
40-60 mol-% amine lipid,

8-10 mol-% neutral lipid; and

2.5-4 mol-% PEG lipid,

wherein the remainder of the lipid component is helper lipid,

wherein the amine lipid is represented by the following structural formula



, and

wherein the N/P ratio of the LNP composition is 5-7.

71. The LNP composition of claim 70, comprising 50-60 mol-% amine lipid.

72. The LNP composition of claim 70 or 71, wherein the helper lipid is

cholesterol.

73. The LNP composition of any one of claims 70-72, wherein the neutral lipid is DSPC.

74. The LNP composition of any one of claims 70-73, wherein the PEG lipid is a PEG-DMG or PEG2k-DMG.

75. The LNP composition of any one of claims 70-74, wherein the mRNA encodes a Cas nuclease, and wherein the RNA component further comprises a guide RNA.

76. The LNP composition of claim 75, wherein the guide RNA is a sgRNA.

77. The LNP composition of any one of claims 70-76, comprising about 50% amine lipid, about 38% helper lipid, about 9% neutral lipid, and about 3% PEG lipid.