STABLE COMPOSITIONS OF MRNA-LOADED LIPID NANOPARTICLES AND PROCESSES OF MAKING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application Serial No. 62/877,597 filed July 23, 2019, the disclosures of which are hereby incorporated by reference.

BACKGROUND

[0002] Messenger RNA therapy (MRT) is becoming an increasingly important approach for the treatment of a variety of diseases. MRT involves administration of messenger RNA (mRNA) to a patient in need of the therapy for production of the protein encoded by the mRNA within the patient's body. Lipid nanoparticles are commonly used to encapsulate mRNA for efficient *in vivo* delivery of mRNA.

[0003] Much effort has been made on developing improved methods and compositions that can enhance *in vivo* delivery and/or expression of mRNA using lipid nanoparticles, which can be adapted to a scalable and cost-effective manufacturing process. At the same time, it is important that any such enhancements to *in vivo* delivery and/or expression of mRNA also maintain or improve upon the safety and tolerability of the compositions associated with lipid mediated mRNA delivery.

SUMMARY ~~OF THE INVENTION~~

[0004] The present ~~invention~~disclosure provides, among other things, further improved compositions and processes for preparing mRNA-loaded lipid nanoparticles (mRNA-LNPs). Prior to the present ~~invention~~disclosure, PEG-modified lipids typically were included in lipid nanoparticle (LNP) formulations as they were known to increase shelf stability and *in vivo* circulating time. On the other hand, PEG-modified lipids may induce accelerated blood clearance (ABC) and/or innate immune response by, among other things, producing anti-PEG antibodies. To address this

issue, attempts have been made to prepare mRNA-LNPs without PEG-modified lipids. However, it has been observed that mRNA-loaded LNPs formed in the absence of PEG-modified lipids or PEG have large and unstable sizes, particularly after freeze and thaw, or tend to precipitate, rendering them unsuitable for therapeutic use. The present ~~invention~~disclosure is, in part, based on the surprising discovery that unexpectedly stable mRNA-loaded LNPs with low or no PEG-modified lipids can be made by mixing the mRNA and lipids in the presence of amphiphilic block copolymer such as poloxamer. As described in more detail below, mRNA-LNPs made according to the present ~~invention~~disclosure have sizes comparable to those conventional LNPs containing a typical amount of PEG-modified lipids and more importantly, are stable following one or more freeze thaw cycles. In particular, the mRNA-LNPs according to the present ~~invention~~disclosure maintain an average diameter within 50%, and in some cases, within 10% of the original average size following one or more freeze thaw cycles. Moreover, poloxamer shielded LNPs with low or no PEG-modified lipids (e.g., < 0.5% PEG-modified lipids) achieved *in vivo* protein expression profile similar to conventional LNPs (e.g., those with 5% PEG-modified lipids). Thus, the present ~~invention~~disclosure provides further improved mRNA-LNPs with exceptional stability and is particularly useful in cases where LNPs comprising low or no PEG-modified lipids are desired, for example, to avoid generating anti-PEG antibodies, and/or ABC.

[0005] In one aspect, the present disclosure provides 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. In one ~~aspect~~example, the present ~~invention~~disclosure provides a stable composition comprising lipid nanoparticles encapsulating messenger RNA (mRNA) encoding a protein or a peptide, wherein each of the lipid nanoparticles comprises one or more cationic lipids and less than 0.5% of PEG-modified lipids or PEG and is stable following one or more freeze thaws. In some ~~embodiment~~examples, the lipid nanoparticles further comprise one or more non-cationic lipids.

[0006] In some ~~embodiment~~examples, the lipid nanoparticles comprise a cationic lipid, dioleoylphosphatidylethanolamine (DOPE) as the non-cationic lipid, and less than about 0.5% of

PEG-modified lipids or PEG. In some ~~embodiment~~examples, the lipid nanoparticles comprise a cationic lipid, 1,2-Dierucoyl-sn-glycero-3-phosphoethanolamine (DEPE) as the non-cationic lipid, and less than about 0.5% of PEG-modified lipids or PEG.

[0007] In some ~~embodiment~~examples, the lipid nanoparticles encapsulating the mRNA maintain an average diameter within 50% of the original average size following one or more freeze thaw cycles. In some ~~embodiment~~examples, the lipid nanoparticles encapsulating the mRNA maintain an

average diameter within 40% of the original average size following one or more freeze thaw cycles. In some ~~embodiment~~examples, the lipid nanoparticles encapsulating the mRNA maintain an average diameter within 30% of the original average size following one or more freeze thaw cycles. In some ~~embodiment~~examples, the lipid nanoparticles encapsulating the mRNA maintain an average diameter within 20% of the original average size following one or more freeze thaw cycles. In some ~~embodiment~~examples, the lipid nanoparticles encapsulating the mRNA maintain an average diameter within 10% of the original average size following one or more freeze thaw cycles. In some ~~embodiment~~examples, the lipid nanoparticles encapsulating the mRNA maintain an average diameter within 5% of the original average size following one or more freeze thaw cycles.

[0008] In some ~~embodiment~~examples, the lipid nanoparticles have an mRNA encapsulation efficiency of between about 50% and 99%. In some ~~embodiment~~examples, the lipid nanoparticles have an mRNA encapsulation efficiency of between about 60% and 90%. In some ~~embodiment~~examples, the lipid nanoparticles have an mRNA encapsulation efficiency of about 60%. In some ~~embodiment~~examples, the lipid nanoparticles have an mRNA encapsulation efficiency of about 70%. In some ~~embodiment~~examples, the lipid nanoparticles have an mRNA encapsulation efficiency of about 80%. In some ~~embodiment~~examples, the lipid nanoparticles have an mRNA encapsulation efficiency of about 90%.

[0009] In some ~~embodiment~~examples, each of the lipid nanoparticles further comprises a cholesterol-based lipid.

[0010] In some ~~embodiment~~examples, each of the lipid nanoparticle comprises 0.4% of PEG-modified lipids or less. In some ~~embodiment~~examples, each of the lipid nanoparticle comprises 0.3% of PEG-modified lipids or less. In some ~~embodiment~~examples, each of the lipid nanoparticle comprises 0.2% of PEG-modified lipids or less. In some ~~embodiment~~examples, each of the lipid nanoparticle comprises 0.1% of PEG-modified lipids or less.

[0011] In some ~~embodiment~~examples, each of the lipid nanoparticle is substantially free of PEG-modified lipids.

[0012] In some ~~embodiment~~examples, each of the lipid nanoparticle comprises an amphiphilic block copolymer.

[0013] In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 3% amphiphilic block copolymer. In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 3% amphiphilic block copolymer. In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 2.5% amphiphilic block copolymer. In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 2% amphiphilic block copolymer. In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 1.5% amphiphilic block copolymer. In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 1% amphiphilic block copolymer. In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 0.5% amphiphilic block copolymer. In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 0.05% amphiphilic block copolymer. In some ~~embodiment~~examples, each of the lipid nanoparticles comprises less than 0.01% amphiphilic block copolymer.

[0014] In some ~~embodiment~~examples, the composition comprises less than 0.05% amphiphilic block copolymer of the total composition by weight. In some ~~embodiment~~examples, the composition comprises less than 0.04% amphiphilic block copolymer of the total composition by weight. In some ~~embodiment~~examples, the composition comprises less than 0.03% amphiphilic block copolymer of the total composition by weight. In some ~~embodiment~~examples, the composition comprises less than 0.02% amphiphilic block copolymer of the total composition by weight. In some ~~embodiment~~examples, the composition comprises less than 0.01% amphiphilic block copolymer of the total composition by weight.

[0015] In some ~~embodiment~~examples, the composition comprises a residual of amphiphilic block copolymer.

[0016] In some ~~embodiment~~examples, a suitable amphiphilic block copolymer is a poloxamer.

[0017] In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 84. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 101. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 105. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 108. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 122. In some ~~embodiment~~examples, t a suitable poloxamer is poloxamer 123. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 124. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 181. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 182. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 183. In

some ~~embodiment~~examples, a suitable poloxamer is poloxamer 184. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 185. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 188. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 212. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 215. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 217. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 231. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 234. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 235. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 237. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 238. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 282. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 284. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 288. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 304. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 331. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 333. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 334. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 335. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 338. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 401. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 402. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 403. In some ~~embodiment~~examples, a suitable poloxamer is poloxamer 407. In some ~~embodiment~~examples, a suitable poloxamer is a combination thereof.

[0018] In ~~one~~ another aspect, the present ~~invention~~disclosure provides 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.

[0019] In ~~one~~ another aspect, the present ~~invention~~disclosure provides 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).

[0020] In some ~~embodiment~~examples, the poloxamer is present in the lipid nanoparticles at an amount of less than 0.1%. In some ~~embodiment~~examples, the poloxamer is present in the lipid nanoparticles at an amount of less than 0.05%.

[0021] In some ~~embodiment~~examples, a suitable non-cationic lipid is dioleoylphosphatidylethanolamine (DOPE). In some ~~embodiment~~examples, a suitable non-cationic lipid is 1,2-Dierucoyl-sn-glycero-3-phosphoethanolamine (DEPE).

[0022] In some ~~embodiment~~examples, each of the lipid nanoparticles does not comprise a cholesterol-based lipid.

[0023] In some ~~embodiment~~examples, each of the lipid nanoparticles is a two-component lipid nanoparticles.

[0024] In some ~~embodiment~~examples, a suitable poloxamer has ethylene oxide units from about 10 to about 150.

[0025] In some ~~embodiment~~examples, a suitable poloxamer has propylene oxide units from about 10 to about 100.

[0026] In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 4,000 g/mol to about 20,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 1,000 g/mol to about 50,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 1,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 2,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 3,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 4,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 5,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 6,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 7,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 8,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 9,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 10,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 20,000 g/mol. In some ~~embodiment~~examples, a

suitable poloxamer has an average molecular weight of about 25,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 30,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 40,000 g/mol. In some ~~embodiment~~examples, a suitable poloxamer has an average molecular weight of about 50,000 g/mol.

[0027] In some ~~embodiment~~examples, the lipid nanoparticles have an average size of less than about 250 nm. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 200 nm or less. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 180 nm or less. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 160 nm or less. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 150 nm or less. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 140 nm or less. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 130 nm or less. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 120 nm or less. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 110 nm or less. In some ~~embodiment~~examples, the lipid nanoparticles have an average size of about 100 nm or less.

[0028] In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.3 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.25 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.20 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.18 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.17 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.16 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.15 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.14 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.13 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.12 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.11 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.10 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.09 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.08 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of

0.07 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.06 or less. In some ~~embodiment~~examples, the lipid nanoparticles have a polydispersity index (PDI) of 0.05 or less.

[0029] In one ~~aspect~~example, the present ~~invention~~disclosure provides, among other things, 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 the present ~~invention~~disclosure.

[0030] In another aspect, the present disclosure provides a use of a stable composition according to any one of the preceding claims in the manufacture of a medicament for treating a subject having a deficiency in a protein or peptide, the use comprising delivery of messenger RNA (mRNA) for *in vivo* production of the protein or the peptide, wherein the stable composition to be administered does not result in anti-PEG antibodies and/or accelerated blood clearance (ABC) in the subject. In one ~~aspect~~example, the present ~~invention~~disclosure provides, among other things, 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 the present ~~invention~~disclosure, wherein the administering the stable composition does not results in anti-PEG antibodies and/or accelerated blood clearance (ABC) in the subject.

[0031] In one ~~aspect~~example, the present ~~invention~~disclosure provides, among other things, 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 the present ~~invention~~disclosure.

[0032] In some ~~embodiment~~examples, the administering the stable composition generates low to no anti-PEG antibodies in the subject.

[0033] In some ~~embodiment~~examples, the administering the stable composition reduces or avoids accelerated blood clearance (ABC) in the subject.

[0034] In some ~~embodiment~~examples, the stable composition is administered by intravenous injection.

[0035] In some ~~embodiment~~examples, the stable composition is administered by pulmonary delivery.

[0036] In some ~~embodiment~~examples, the stable composition is administered by intramuscular delivery.

[0037] In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 6 hours after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 12 hours after

administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 18 hours after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 24 hours after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 30 hours after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 36 hours after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 48 hours after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 72 hours after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 5 days after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 1 week after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 2 weeks after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 3 weeks after administration. In some ~~embodiment~~examples, the administering of the stable composition results in expression of the protein or the peptide encoded by the mRNA for at least about 4 weeks after administration.

[0038] In ~~one~~ another aspect, the present ~~invention~~disclosure provides, among other things, 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.

[0039] In some ~~embodiment~~examples, 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.

[0040] In some ~~embodiment~~examples, the lipid solution comprises pre-formed lipid nanoparticles.

[0041] In some ~~embodiment~~examples, wherein the mRNA solution and/or the lipid solution are at a pre-determined temperature higher than ambient temperature.

[0042] In some ~~embodiment~~examples, the poloxamer is first added to the mRNA solution.

[0043] In some ~~embodiment~~examples, the poloxamer is present in an amount lower than its critical micelle concentration (CMC).

[0044] In some ~~embodiment~~examples, the poloxamer is present in an amount about 1% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 2% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 3% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 4% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 5% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 6% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 7% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 8% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 9% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 10% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 15% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 20% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 25% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 30% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 35% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 40% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 45% lower than its CMC. In some ~~embodiment~~examples, the poloxamer is present in an amount about 50% lower than its CMC.

[0045] In some ~~embodiment~~examples, the process further comprises a step of removing the poloxamer.

[0046] In some ~~embodiment~~examples, the poloxamer is removed by dialysis.

[0047] In some ~~embodiment~~examples, less than about 0.1% poloxamer remains upon removal. In some ~~embodiment~~examples, less than about 0.05% poloxamer remains upon removal. In some ~~embodiment~~examples, less than about 0.01% poloxamer remains upon removal.

[0048] In some ~~embodiment~~examples, a residual amount of poloxamer remains upon removal.

[0049] In some ~~embodiment~~examples, the amount of poloxamer remaining after removal is undetectable.

[0050] In some ~~embodiment~~examples, the process does not include mixing any cholesterol lipids.

[0051] In ~~one~~ another aspect, the present ~~invention~~disclosure provides, among other things, a composition comprising lipid nanoparticles encapsulating mRNA formed according to a process disclosed herein.

[0052] In this application, the use of "or" means "and/or" unless stated otherwise. As used in this disclosure, the term "comprise" and variations of the term, such as "comprising" and "comprises," are not intended to exclude other additives, components, integers or steps. As used in this application, the terms "about" and "approximately" are used as equivalents. Both terms are meant to cover any normal fluctuations appreciated by one of ordinary skill in the relevant art.

[0053] Other features, objects, and advantages of the present ~~invention~~disclosure are apparent in the detailed description, drawings and claims that follow. It should be understood, however, that the detailed description, the drawings, and the claims, while indicating ~~embodiment~~examples of the present ~~invention~~disclosure, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the ~~invention~~disclosure will become apparent to those skilled in the art.

BRIEF DESCRIPTION OF THE DRAWING

[0054] The following figures are for illustration purposes only and not for limitation.

[0055] **Figure 1** shows a schematic of an exemplary LNP-mRNA encapsulation process that involves mixing an aqueous solution comprising mRNA and poloxamer with a lipid solution using a pump system to generate mRNA-LNPs in a LNP formation solution and then exchanging the LNP formation solution for a drug product formulation solution.

CLAIMS

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; optionally,
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; or further optionally 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.

~~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.~~

4~~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%.

5~~7~~. The stable composition of any one of the preceding claims, wherein each of the lipid nanoparticles further comprises a cholesterol-based lipid.

68. 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, or substantially free of PEG-modified lipids.

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.

712. The stable composition of any one of the preceding claims, claim 11, wherein each of the lipid nanoparticles comprises a poloxamer, and wherein the stable composition comprises less than 0.05 %, less than 0.04%, less than 0.03%, less than 0.02%, or less than 0.01% poloxameramphiphilic 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.~~

8~~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.

9~~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).

10~~18~~. The stable composition of claim 8 or claim 9~~17~~, wherein the poloxamer is present in the lipid nanoparticles at an amount of less than 0.1%, or at an amount of at less than 0.05%.

~~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%.~~

11~~20~~. The stable composition of any one of claims 8-10~~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.~~

12~~36~~. ~~A method for delivery of messenger RNA (mRNA) for *in vivo* production of a protein or a peptide, comprising administering to a subject~~ Use of a stable composition according to any one of the preceding claims in the manufacture of a medicament for treating a subject having a deficiency in a protein or peptide, the use comprising delivery of messenger RNA (mRNA) for *in vivo* production of the protein or the peptide, wherein ~~the administering~~ the stable composition to be administered does not result~~s~~ 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.~~

1342. 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.

1443. The process of claim 4213, 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.

1547. The process of claim 13 or claim 14any one of claims 42-46, wherein the poloxamer is present in an amount lower than its critical micelle concentration (CMC), optionally, wherein the poloxamer is present in an amount -1%, 2%, 3%, 4%,-5%, -6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% lower than its 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.

1650. The process of any one of claims 13-1542-49, wherein the process further comprises a step of removing the poloxamer, optionally, wherein the poloxamer is removed by dialysis.

~~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.~~

17~~53~~. The process of claim 16~~52~~, wherein less than about 0.01% poloxamer remains upon removal.

18~~54~~. The process of claim 17~~52~~, wherein a residual amount of poloxamer remains upon removal.

19~~55~~. The process of any one of claims 16-18~~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.~~

20~~66~~. A composition comprising lipid nanoparticles encapsulating mRNA formed according to a process of any one of claims 13-19~~42-63~~.