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 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 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 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 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 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 composition comprises a poloxamer at less than 0.05% by weight of the total composition. In one example, the present 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 examples, the lipid nanoparticles further comprise one or more non-cationic lipids.

[0006] In some 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 examples, the lipid nanoparticles comprise a cationic

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

[0018] In another example, the present 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 another example, the present 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).

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

[0029] In one example, the present 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 disclosure.

[0030] In another aspect, the present disclosure provides a use of a composition according to any one of the preceding claims in the manufacture of a medicament for treating a disease due to a deficiency in a protein or peptide in a subject, wherein the composition is to be administered in the subject, such that the mRNA comprised within the composition results in the *in vivo* production of the protein or peptide. In one example, the present 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 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 example, the present 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 disclosure.

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

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

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

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

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

administration. In some 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 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 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 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 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 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 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 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 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 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 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 another example, the present 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. In another aspect, the present disclosure provides a process of encapsulating messenger RNA (mRNA) in lipid nanoparticles in a composition according to the disclosure, comprising a step of mixing an mRNA solution and a lipid solution in the presence of a poloxamer and a step of removing the poloxamer wherein less than 0.05% of the poloxamer remains upon removal.

[0039] In some 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 examples, the lipid solution comprises pre-formed lipid nanoparticles.

CLAIMS

1. A 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 composition comprises a poloxamer at less than 0.05% by weight of the total composition.

2. The composition of claim 1, wherein the one or more non-cationic lipids comprise dioleoylphosphatidylethanolamine (DOPE).

3. The 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 maintain 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 maintain an average diameter within 5% of the original average size following one or more freeze thaw cycles.

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

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

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

7. The composition of any one of the preceding claims, wherein the composition comprises less than 0.04%, less than 0.03%, less than 0.02%, or less than 0.01% of the poloxamer by weight of the total composition.

8. The composition of any one of the preceding claims, wherein the composition is free of the PEG-modified lipids or PEG.

9. The composition of any one of the preceding claims, wherein the composition comprises a residual amount of the poloxamer.

10. The composition of any one of the preceding claims, wherein the poloxamer has an average molecular weight of 4,000 g/mol to 20,000 g/mol.

11. The composition of any one of the preceding claims, 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 a combination thereof.

12. Use of a composition according to any one of the preceding claims in the manufacture of a medicament for treating a disease due to a deficiency in a protein or peptide in a subject, wherein the composition is to be administered in the subject, such that the mRNA comprised within the composition results in the *in vivo* production of the protein or peptide.

13. A process of encapsulating messenger RNA (mRNA) in lipid nanoparticles in a composition according to any one of claims 1 to 11, comprising a step of mixing an mRNA solution and a lipid solution in the presence of a poloxamer and a step of removing the poloxamer wherein less than 0.05% of the poloxamer remains upon removal.

14. The process of claim 13, 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.

15. The process of claim 13 or 14, 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, before removal.

16. The process of any one of claims 13-15, wherein the poloxamer is removed by dialysis.

17. The process of any one of claims 13-16, wherein less than 0.01% of the poloxamer remains upon removal.

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

19. The process of claim 17, wherein the amount of the poloxamer remaining after removal is undetectable.

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