COMPOSITIONS AND METHODS FOR STABILIZATION OF LIPID NANOPARTICLE MRNA VACCINES

Background

[1] Messenger RNA (mRNA) is proving to be an exciting therapeutic modality and has garnered significant recent attention, particularly in the vaccine space.

Summary

[2] The present disclosure provides technologies relating to formulation of RNA (*e.g.*, mRNA) therapeutics, and particular to lipid nanoparticle (LNP) formulations comprising RNA (*e.g.*, mRNA) payloads. Among other things, the present disclosure provides therapeutic RNA formulations (*i.e.*, LNP formulations) that are amenable (*e.g.*, stable) to storage and/or handling at temperatures above about -80ºC, or even above about -70ºC, about -60ºC, about -50ºC, about -40ºC, about -30ºC, or about -20ºC. In some ~~embodiment~~examples, provided formulations may be amenable to storage and/or handling at temperatures above freezing (e.g, above about 0ºC), at standard refrigeration temperature (*e.g.*, within a range of about 1ºC to about 8ºC, or about 2ºC to about 8ºC, or about 2ºC to about 6ºC, or about 2ºC to about 4ºC), and/or at room temperature (*e.g.*, within a range of about 15ºC to about 25ºC, or about 20ºC to about 23ºC).

[3] In some ~~embodiment~~examples, the present disclosure provides formulations that are amendable to drying and/or that are dry (*e.g.*, that are lyophilized formulations).

[4] The present disclosure particularly provides certain formulations useful as (and/or in the preparation of) vaccines.

[5] In some ~~embodiment~~examples, the present disclosure provides formulations (and specifically LNP formulations) of RNA encoding a viral antigen (*e.g.*, a SARS-CoV2 antigen such as an S-protein or epitope thereof). Specific exemplified formulations include an RNA construct that is a BNT162 construct (*e.g.*, as described in Walsh, E. *et al.* RNA-Based COVID-19 Vaccine BNT162b2 Selected for a Pivotal Efficacy Study. *medRxiv* (2020)), *e.g.*, BNT162b2; and PCT Application Number No. PCT/EP2020/081981 filed November 12, 2020 and entitled

"Coronavirus Vaccine", the contents of each of which are incorporated herein by reference for purposes described herein.)

[5a] In one aspect, the present disclosure provides a formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid; b) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; and c) Tris buffer, wherein the Tris buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM. In another aspect, the present disclosure provides a method of preparing a formulation of as described herein, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) Tris buffer, wherein the Tris buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM; and ii) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v. In yet another aspect, the present disclosure provides a dosage form of a formulation, wherein the formulation comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) mRNA at a concentration of about 0.5 mg/ml; ii) a neutral lipid; iii) a polymer-conjugated lipid; iv) a cationic lipid; v) a steroid; b) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; c) Tris buffer, wherein the Tris buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM; wherein the dosage form is diluted from the formulation.

[5b] In a further aspect, the present disclosure provides a formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid; and b) sucrose at a concentration of about 10% w/v;trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of

about 5% w/v; and c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM; or HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM. In another aspect, the present disclosure provides a method of preparing a formulation as described herein, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM; or HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM; and ii) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v. In another aspect, the present disclosure provides a dosage form of a formulation, wherein the formulation comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) mRNA at a concentration of about 0.5 mg/ml; ii) a neutral lipid; iii) a polymer-conjugated lipid; iv) a cationic lipid; v) a steroid; b) sucrose at a concentration of about 10% w/v; trehalose at a concentration of about 10% w/v; or sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/; and d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM; or HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM; wherein the dosage form is diluted from the formulation.

[5c] In another aspect, the present disclosure provides a formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid; and b) sucrose at a concentration of about 10% w/v; and c) PBS buffer, wherein the PBS buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride. In yet another aspect, the present disclosure provides a method of preparing a formulation as described herein, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a

steroid; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) PBS buffer, wherein the PBS buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride; and ii) sucrose at a concentration of about 10% w/v. In yet another aspect, the present disclosure provides a dosage form of a formulation, wherein the formulation comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) mRNA at a concentration of about 0.5 mg/ml; ii) a neutral lipid; iii) a polymer-conjugaed lipid; iv) a cationic lipid; v) a steroid; b) sucrose at a concentration of about 10% w/v; and c) PBS buffer, wherein the PBS buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride; wherein the dosage form is dilution from the formulation. In yet another aspect, the present disclosure provides a method of preparing a formulation as described herein, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; and ii) sucrose at a concentration of about 10% w/v.

[5d] In another aspect, the present disclosure provides a dosage form of a formulation, wherein the formulation comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) mRNA at a concentration of about 0.5 mg/ml; ii) a neutral lipid; iii) a polymer-conjugated lipid; iv) a cationic lipid; v) a steroid; b) sucrose at a concentration of about 10% w/v; and c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; wherein the dosage form is diluted into the formulation. In yet another aspect, the present disclosure provides a method of preparing a formulation as described hereine, the method comprising steps of: a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises: i) a payload that comprises one or more mRNAs; ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid; and b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises: i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; and ii) sucrose at a concentration of about 10% w/v wherein the first buffer system comprises sucrose at a concentration of about 10% w/v.

[5e] In another aspect, the present disclosure provides a formulation as described herein or a dosage form as described herein for use in delivering a nucleic acid into a cell in a subject. In

another aspect, the present disclosure provides a formulation as described herein or a dosage form as described herein for use in inducing an immune response in a subject. In another aspect, the present disclosure provides a dose of the formulation as described herein, which dose includes 1 µg to 100 µg of mRNA. In another aspect, the present disclosure provides a dose of the formulation as described herein, which dose includes less than 60 µg of mRNA.

[6] In one ~~aspect~~example, a formulation provided herein comprises: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) sucrose at a concentration of about 10% w/v in the formulation; and (c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[7] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) sucrose at a concentration of about 10% w/v in the formulation; and (c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[8] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) sucrose at a concentration of about 10% w/v in the formulation before drying; and (c) Tris buffer, wherein the Tris buffer is

substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[9] Methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) sucrose at a concentration of about 10% w/v in the formulation.

[10] In some ~~embodiment~~examples, a method comprising a step of:

administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v;

c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

wherein the formulation is diluted into the dosage form prior to administration.

[11] In one ~~aspect~~example, provided herein is a formulation comprising: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: (i) a payload that is or comprises one or more mRNAs; (ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) trehalose at a concentration of about 10% w/v in the formulation; and (c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[12] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) trehalose at a concentration of about 10% w/v in the formulation; and c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[13] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; (b) trehalose at a concentration of about 10% w/v in the formulation before drying; and (c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[14] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) trehalose at a concentration of about 10% w/v in the formulation.

[15] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) trehalose at a concentration of about 10% w/v;

c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[16] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation; c) trehalose at a concentration of about 5% w/v in the formulation; d) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[17] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation; c) trehalose at a concentration of about 5% w/v in the formulation; d) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[18] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b)sucrose at a concentration of about 5% w/v in the formulation before drying; c) trehalose at a concentration of about 5% w/v in the formulation before drying; d) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[19] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

(a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

ii) sucrose at a concentration of about 5% w/v in the formulation; and

iii) trehalose at a concentration of about 5% w/v in the formulation.

[20] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[21] In one ~~aspect~~example, a formulation provided herein comprises: (a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

[22] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

[23] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b)sucrose at a concentration of about 10% w/v in the formulation before drying; c) Tris buffer, wherein the Tris

buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[24] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) sucrose at a concentration of about 10% w/v in the formulation.

[25] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v in the formulation;

c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[26] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) trehalose at a concentration of about 10% w/v in the formulation; c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

[27] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) trehalose at a concentration of about 10% w/v in the formulation; c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

[28] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b)trehalose at a concentration of about 10% w/v in the formulation before drying; c) Tris buffer, wherein the Tris

buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[29] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) trehalose at a concentration of about 10% w/v in the formulation.

[30] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) trehalose at a concentration of about 10% w/v in the formulation;

c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[31] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation; c) trehalose at a concentration of about 5% w/v in the formulation; d) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

[32] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation; c) trehalose at a concentration of about 5% w/v in the formulation; d) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

[33] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation before drying; c) trehalose at a concentration

of about 5% w/v in the formulation before drying; d) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[34] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation;

ii) sucrose at a concentration of about 5% w/v in the formulation; and

iii) trehalose at a concentration of about 5% w/v in the formulation.

[35] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[36] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[37] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[38] In some ~~embodiment~~examples, a formulation provided herein is a a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-

ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b)sucrose at a concentration of about 10% w/v in the formulation before drying; c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[39] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) sucrose at a concentration of about 10% w/v in the formulation.

[40] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v in the formulation;

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[41] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) trehalose at a concentration of about 10% w/v in the formulation; c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[42] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) trehalose at a concentration of about 10% w/v in the formulation; c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[43] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at

relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) trehalose at a concentration of about 10% w/v in the formulation before drying; c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[44] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) trehalose at a concentration of about 10% w/v in the formulation.

[45] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) trehalose at a concentration of about 10% w/v in the formulation;

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[46] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation; c) trehalose at a concentration of about 5% w/v in the formulation; d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[47] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation; c) trehalose at a concentration of about 5% w/v in the formulation; d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[48] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-

ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b)sucrose at a concentration of about 5% w/v in the formulation before drying; c) trehalose at a concentration of about 5% w/v in the formulation before drying; d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[49] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

ii) sucrose at a concentration of about 5% w/v in the formulation; and

iii) trehalose at a concentration of about 5% w/v in the formulation.

[50] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[51] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[52] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[53] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or

comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation before drying; c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[54] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) sucrose at a concentration of about 10% w/v in the formulation.

[55] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v in the formulation;

d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[56] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) trehalose at a concentration of about 10% w/v in the formulation; c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[57] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) trehalose at a concentration of about 10% w/v in the formulation; c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[58] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-

6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b)trehalose at a concentration of about 10% w/v in the formulation before drying; c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[59] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) trehalose at a concentration of about 10% w/v in the formulation.

[60] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) trehalose at a concentration of about 10% w/v in the formulation;

d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[61] In one ~~aspect~~example, a formulationprovided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation; c) trehalose at a concentration of about 5% w/v in the formulation; d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[62] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation; c) trehalose at a concentration of about 5% w/v in the formulation; d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

[63] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or

comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 5% w/v in the formulation before drying; c) trehalose at a concentration of about 5% w/v in the formulation before drying; d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

[64] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

ii) sucrose at a concentration of about 5% w/v in the formulation; and

iii) trehalose at a concentration of about 5% w/v in the formulation.

[65] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; wherein the formulation is diluted into the dosage form prior to administration.

[66] In one ~~aspect~~example, a formulation provided herein comprises: a)a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) PBS buffer, wherein the PBS buffer is substantially free of sodium chloride.

[67] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) PBS buffer, wherein the PBS buffer is substantially free of sodium chloride.

[68] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation before drying; c) PBS buffer, wherein the PBS buffer is substantially free of sodium chloride.

[69] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein the PBS buffer is substantially free of sodium chloride; and

ii) sucrose at a concentration of about 10% w/v in the formulation.

[70] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v;

c) PBS buffer, wherein the PBS buffer is substantially free of sodium chloride; wherein the formulation is diluted into the dosage form prior to administration.

[71] In one ~~aspect~~example, a formulation provided herein comprises: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride in the formulation.

[72] In some ~~embodiment~~examples, a formulation provided herein is a frozen formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b) sucrose at a concentration of about 10% w/v in the formulation; c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride in the formulation.

[73] In some ~~embodiment~~examples, a formulation provided herein is a dry formulation comprising: a) a lipid nanoparticle (LNP), wherein the LNP comprises: i) a payload that is or

comprises one or more mRNAs; ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and b)sucrose at a concentration of about 10% w/v in the formulation before drying; c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride in the formulation before drying.

[74] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride in the formulation; and

ii) sucrose at a concentration of about 10% w/v in the formulation.

[75] In some ~~embodiment~~examples, provided herein is a method comprising a step of administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v in the formulation;

c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride; wherein the formulation is diluted into the dosage form prior to administration.

[76] In some ~~embodiment~~examples, methods of providing such formulations described herein are also described herein. In some ~~embodiment~~examples, provided herein is a method of preparing a formulation comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride in the formulation; and

ii) sucrose at a concentration of about 10% w/v in the formulation wherein the first buffer system comprises sucrose at a concentration of about 10% w/v.

[77] In some ~~embodiment~~examples, provided herein is a method of delivering a nucleic acid into a cell in a subject comprising a step of administering a formulation as described in any of the preceding claims.

[78] In some ~~embodiment~~examples, provided herein is a method of inducing an immune response in a subject comprising a step of administering to the subject a formulation as described in any of the preceding claims.

Definitions

[79] In this application, unless otherwise clear from context, (i) the term "a" may be understood to mean "at least one"; (ii) the term "or" may be understood to mean "and/or"; (iii) the terms "comprising" and "including" may be understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) the terms "about" and "approximately" may be understood to permit standard variation as would be understood by those of ordinary skill in the art; and (v) where ranges are provided, endpoints are included.

[80] **Administration:** As used herein, the term "administration" refers to the administration of a composition to a subject. Exemplary routes of administration may include bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal and vitreal. In many embodiments, provided technologies relate to LNP compositions (*e.g.*, comprising a BNT162 construct) that are administered by intramuscular injection. In some embodiments, LNP compositions are administered in a first administration followed by one or more administrations (*e.g.*, one or more booster administrations). In some embodiments, a period of time, *e.g.*, about 24, 48, 72, 96 hours or more, including for about 1, 2, 3, 4, or more weeks, separates each administration of an LNP compositions, *e.g.*, between a first administration and a second administration. In some embodiments, a period of time separating administrations is about 3 weeks (*e.g.*, about 21 days).

[81] **Antibody agent:** As used herein, the term "antibody agent" refers to an agent that specifically binds to a particular antigen. In some embodiments, the term encompasses any polypeptide or polypeptide complex that includes immunoglobulin structural elements sufficient

Claims

We claim:

1. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; ~~and~~

b) sucrose at a concentration of about 10% w/v ~~in the formulation~~;
trehalose at a concentration of about 10% w/v; or
sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; and

c) Tris buffer, wherein the Tris buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM ~~in the formulation~~.

2. The formulation of claim 1, wherein the formulation is a frozen formulation.

~~2. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 10% w/v in the formulation;~~

~~c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~3. A dry formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 10% w/v in the formulation before drying;~~

~~c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.~~

~~4~~3. A method of preparing a formulation of claim 1, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM ~~in the formulation~~; and

ii) sucrose at a concentration of about 10% w/v ~~in the formulation;~~ trehalose at a concentration of about 10% w/v; or

sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v.

~~5. The method of claim 4, further comprising a step of:~~

~~c) freezing the formulation.~~

~~6. The method of claim 4, further comprising a step of:~~

~~c) drying the formulation.~~

~~7. A method of preparing a dosage form, the method comprising a step of:~~

~~a) diluting a formulation of claim 1;~~

~~b) thawing and diluting a formulation of claim 2; and/or~~

~~c) resuspending and diluting a formulation of claim 3.~~

~~8. The method of claim 7, further comprising administering the dosage form to a subject in need of thereof.~~

~~9. The method of claim 8, wherein the subject is in need of an expression product of the mRNA.~~

~~104~~. ~~A method comprising a step of:~~

~~administering a~~ A dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) a neutral lipid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml~~;

iii) a polymer-conjugated lipid~~2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml~~;

iv) a cationic lipid~~distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml~~;

v) a steroid~~cholesterol at a concentration of about 3.1 mg/ml~~;

b) sucrose at a concentration of about 10% w/v;

trehalose at a concentration of about 10% w/v; or

sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v;

c) Tris buffer, wherein the Tris buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride, and is at a concentration of about 10 mM ~~in the formulation~~;

wherein the ~~formulation is diluted into the~~ dosage form is diluted from the formulation~~prior to administration~~.

~~11~~5. ~~A~~ The formulation of claim 1, comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) ~~trehalose~~ sucrose at a concentration of about 10% w/v ~~in the formulation~~; and

c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM ~~in the formulation~~.

~~12. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) trehalose at a concentration of about 10% w/v in the formulation;~~

~~c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~13. A dry formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) trehalose at a concentration of about 10% w/v in the formulation before drying;~~

~~c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.~~

~~14~~6. ~~A~~ The method of ~~preparing a formulation~~claim 3, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM ~~in the formulation~~; and

ii) ~~trehalose~~ sucrose at a concentration of about 10% w/v ~~in the formulation~~.

~~15. The method of claim 14, further comprising a step of:~~

c) freezing the formulation.

16. The method of claim 14, further comprising a step of:

c) drying the formulation.

17. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 11;

b) thawing and diluting a formulation of claim 12; and/or

c) resuspending and diluting a formulation of claim 13.

18. The method of claim 17, further comprising administering the dosage form to a subject in need of thereof.

19. The method of claim 18, wherein the subject is in need of an expression product of the mRNA.

20. A The method dosage form of claim 4, comprising a step of:

administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) a neutral lipid((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) a polymer-conjugated lipid2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) a cationic lipiddistearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) a steroidcholesterol at a concentration of about 3.1 mg/ml;

b) trehalose sucrose at a concentration of about 10% w/v; and

c) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

wherein the dosage form is diluted from the formulation ~~is diluted into the dosage form prior to administration~~.

~~21. A formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 5% w/v in the formulation;~~

~~c) trehalose at a concentration of about 5% w/v in the formulation;~~

~~d) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~22. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 5% w/v in the formulation;~~

~~c) trehalose at a concentration of about 5% w/v in the formulation;~~

~~d) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~23. A dry formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 5% w/v in the formulation before drying;

c) trehalose at a concentration of about 5% w/v in the formulation before drying;

d) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

24. A method of preparing a formulation, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

ii) sucrose at a concentration of about 5% w/v in the formulation; and

iii) trehalose at a concentration of about 5% w/v in the formulation.

25. The method of claim 24, further comprising a step of:

c) freezing the formulation.

26. The method of claim 24, further comprising a step of:

c) drying the formulation.

27. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 21;

b) thawing and diluting a formulation of claim 22; and/or

c) resuspending and diluting a formulation of claim 23.

28. The method of claim 27, further comprising administering the dosage form to a subject in need of thereof.

29. The method of claim 28, wherein the subject is in need of an expression product of the mRNA.

30. A method comprising a step of:

administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

wherein the formulation is diluted into the dosage form prior to administration.

31. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v in the formulation;

c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

32. A frozen formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v in the formulation;

c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

33. A dry formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v in the formulation before drying;

c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

34. A method of preparing a formulation comprising steps of:

~~a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:~~

~~i) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation; and~~

~~ii) sucrose at a concentration of about 10% w/v in the formulation.~~

~~35. The method of claim 34, further comprising a step of:~~

~~c) freezing the formulation.~~

~~36. The method of claim 34, further comprising a step of:~~

~~c) drying the formulation.~~

~~37. A method of preparing a dosage form, the method comprising a step of:~~

~~a) diluting a formulation of claim 31;~~

~~b) thawing and diluting a formulation of claim 32; and/or~~

~~c) resuspending and diluting a formulation of claim 33.~~

~~38. The method of claim 37, further comprising administering the dosage form to a subject in need of thereof.~~

~~39. The method of claim 38, wherein the subject is in need of an expression product of the mRNA.~~

~~40. A method comprising a step of:~~

~~administering a dosage form of a formulation, wherein the formulation comprises:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) mRNA at a concentration of about 0.5 mg/ml;~~

~~ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;~~

~~iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;~~

~~iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;~~

~~v) cholesterol at a concentration of about 3.1 mg/ml;~~

~~b) sucrose at a concentration of about 10% w/v in the formulation;~~

~~c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation;~~

~~wherein the formulation is diluted into the dosage form prior to administration.~~

~~41. A formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) trehalose at a concentration of about 10% w/v in the formulation;~~

~~c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~42. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide~~

(ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) trehalose at a concentration of about 10% w/v in the formulation;

c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

43. A dry formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) trehalose at a concentration of about 10% w/v in the formulation before drying;

c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

44. A method of preparing a formulation, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) trehalose at a concentration of about 10% w/v in the formulation.

45. The method of claim 44, further comprising a step of:

c) freezing the formulation.

46. The method of claim 44, further comprising a step of:

c) drying the formulation.

47. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 41;

b) thawing and diluting a formulation of claim 42; and/or

c) resuspending and diluting a formulation of claim 43.

48. The method of claim 47, further comprising administering the dosage form to a subject in need of thereof.

49. The method of claim 48, wherein the subject is in need of an expression product of the mRNA.

50. A method comprising a step of:

administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) trehalose at a concentration of about 10% w/v in the formulation;

c) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation;

wherein the formulation is diluted into the dosage form prior to administration.

51. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

52. A frozen formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation.

53. A dry formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide

(ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 5% w/v in the formulation before drying;

c) trehalose at a concentration of about 5% w/v in the formulation before drying;

d) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

54. A method of preparing a formulation, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation;

ii) sucrose at a concentration of about 5% w/v in the formulation; and

iii) trehalose at a concentration of about 5% w/v in the formulation.

55. The method of claim 54, further comprising a step of:

c) freezing the formulation.

56. The method of claim 54, further comprising a step of:

c) drying the formulation.

57. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 51;

b) thawing and diluting a formulation of claim 52; and/or

~~c) resuspending and diluting a formulation of claim 53.~~

~~58. The method of claim 57, further comprising administering the dosage form to a subject in need of thereof.~~

~~59. The method of claim 58, wherein the subject is in need of an expression product of the mRNA.~~

~~60. A method comprising a step of:~~

~~administering a dosage form of a formulation, wherein the formulation comprises:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) mRNA at a concentration of about 0.5 mg/ml;~~

~~ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;~~

~~iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;~~

~~iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;~~

~~v) cholesterol at a concentration of about 3.1 mg/ml;~~

~~b) sucrose at a concentration of about 5% w/v in the formulation;~~

~~c) trehalose at a concentration of about 5% w/v in the formulation;~~

~~d) Tris buffer, wherein the Tris buffer comprises about 6 mg/ml sodium chloride and is at a concentration of about 10 mM in the formulation;~~

~~wherein the formulation is diluted into the dosage form prior to administration.~~

~~6~~18. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-~~

~~ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) sucrose at a concentration of about 10% w/v ~~in the formulation~~;
trehalose at a concentration of about 10% w/v; or
sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v; and

c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM ~~in the formulation~~; or
HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM.

~~62. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 10% w/v in the formulation;~~

~~c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~63. A dry formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 10% w/v in the formulation before drying;~~

~~c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.~~

~~64~~9. A method of preparing a formulation of claim 8, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM ~~in the formulation~~; or
HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM; and

ii) sucrose at a concentration of about 10% w/v ~~in the formulation~~;
trehalose at a concentration of about 10% w/v; or
sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/v.

~~65. The method of claim 64, further comprising a step of:~~

~~c) freezing the formulation.~~

~~66. The method of claim 64, further comprising a step of:~~

~~c) drying the formulation.~~

~~67. A method of preparing a dosage form, the method comprising a step of:~~

~~a) diluting a formulation of claim 61;~~

~~b) thawing and diluting a formulation of claim 62; and/or~~

~~c) resuspending and diluting a formulation of claim 63.~~

~~68. The method of claim 67, further comprising administering the dosage form to a subject in need of thereof.~~

~~69. The method of claim 68, wherein the subject is in need of an expression product of the mRNA.~~

~~70~~10. ~~A method comprising a step of:~~

~~administering a~~A dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) a neutral lipid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml~~;

iii) a polymer-conjugated lipid~~2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml~~;

iv) a cationic lipid~~distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml~~;

v) a steroid~~cholesterol at a concentration of about 3.1 mg/ml~~;

b) sucrose at a concentration of about 10% w/v ~~in the formulation~~;

trehalose at a concentration of about 10% w/v; or

sucrose at a concentration of about 5% w/v, and trehalose at a concentration of about 5% w/; and

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM ~~in the formulation~~; or

HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM;

wherein the dosage form is diluted from the formulation ~~is diluted into the dosage form prior to administration~~.

~~71. A formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) trehalose at a concentration of about 10% w/v in the formulation;~~

~~c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~72. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) trehalose at a concentration of about 10% w/v in the formulation;~~

~~c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~73. A dry formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) trehalose at a concentration of about 10% w/v in the formulation before drying;~~

c) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

74. A method of preparing a formulation, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) trehalose at a concentration of about 10% w/v in the formulation.

75. The method of claim 74, further comprising a step of:

c) freezing the formulation.

76. The method of claim 74, further comprising a step of:

c) drying the formulation.

77. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 71;

b) thawing and diluting a formulation of claim 72; and/or

c) resuspending and diluting a formulation of claim 73.

78. The method of claim 77, further comprising administering the dosage form to a subject in need of thereof.

79. The method of claim 78, wherein the subject is in need of an expression product of the mRNA.

80. A method comprising a step of:

administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) trehalose at a concentration of about 10% w/v in the formulation;

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

wherein the formulation is diluted into the dosage form prior to administration.

81. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

82. A frozen formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

83. A dry formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 5% w/v in the formulation before drying;

c) trehalose at a concentration of about 5% w/v in the formulation before drying;

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

84. A method of preparing a formulation, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide

(ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

ii) sucrose at a concentration of about 5% w/v in the formulation; and

iii) trehalose at a concentration of about 5% w/v in the formulation.

85. The method of claim 84, further comprising a step of:

c) freezing the formulation.

86. The method of claim 84, further comprising a step of:

c) drying the formulation.

87. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 81;

b) thawing and diluting a formulation of claim 82; and/or

c) resuspending and diluting a formulation of claim 83.

88. The method of claim 87, further comprising administering the dosage form to a subject in need of thereof.

89. The method of claim 88, wherein the subject is in need of an expression product of the mRNA.

90. A method comprising a step of:

administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 5% w/v in the formulation;

c) trehalose at a concentration of about 5% w/v in the formulation;

d) His buffer, wherein the His buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

wherein the formulation is diluted into the dosage form prior to administration.

91. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v in the formulation;

c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

92. A frozen formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v in the formulation;

c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

93. A dry formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) sucrose at a concentration of about 10% w/v in the formulation before drying;

c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

94. A method of preparing a formulation, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) sucrose at a concentration of about 10% w/v in the formulation.

95. The method of claim 94, further comprising a step of:

c) freezing the formulation.

96. The method of claim 94, further comprising a step of:

c) drying the formulation.

97. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 91;

b) thawing and diluting a formulation of claim 92; and/or

c) resuspending and diluting a formulation of claim 93.

98. The method of claim 97, further comprising administering the dosage form to a subject in need of thereof.

99. The method of claim 98, wherein the subject is in need of an expression product of the mRNA.

100. A method comprising a step of:

administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;

iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;

iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;

v) cholesterol at a concentration of about 3.1 mg/ml;

b) sucrose at a concentration of about 10% w/v in the formulation;

d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

wherein the formulation is diluted into the dosage form prior to administration.

101. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) trehalose at a concentration of about 10% w/v in the formulation;

c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

102. A frozen formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) trehalose at a concentration of about 10% w/v in the formulation;

c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.

103. A dry formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) trehalose at a concentration of about 10% w/v in the formulation before drying;

c) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.

104. A method of preparing a formulation, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that is or comprises one or more mRNAs;

ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation; and

ii) trehalose at a concentration of about 10% w/v in the formulation.

105. The method of claim 104, further comprising a step of:

c) freezing the formulation.

106. The method of claim 104, further comprising a step of:

c) drying the formulation.

107. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 101;

b) thawing and diluting a formulation of claim 102; and/or

c) resuspending and diluting a formulation of claim 103.

108. The method of claim 107, further comprising administering the dosage form to a subject in need of thereof.

~~109. The method of claim 108, wherein the subject is in need of an expression product of the mRNA.~~

~~110. A method comprising a step of:~~

~~administering a dosage form of a formulation, wherein the formulation comprises:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) mRNA at a concentration of about 0.5 mg/ml;~~

~~ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;~~

~~iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;~~

~~iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;~~

~~v) cholesterol at a concentration of about 3.1 mg/ml;~~

~~b) trehalose at a concentration of about 10% w/v in the formulation;~~

~~d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;~~

~~wherein the formulation is diluted into the dosage form prior to administration.~~

~~111. A formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 5% w/v in the formulation;~~

~~c) trehalose at a concentration of about 5% w/v in the formulation;~~

~~d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~112. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 5% w/v in the formulation;~~

~~c) trehalose at a concentration of about 5% w/v in the formulation;~~

~~d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation.~~

~~113. A dry formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 5% w/v in the formulation before drying;~~

~~c) trehalose at a concentration of about 5% w/v in the formulation before drying;~~

~~d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation before drying.~~

~~114. A method of preparing a formulation, the method comprising steps of:~~

~~a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide~~

(ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;

ii) sucrose at a concentration of about 5% w/v in the formulation; and

iii) trehalose at a concentration of about 5% w/v in the formulation.

115. The method of claim 114, further comprising a step of:

c) freezing the formulation.

116. The method of claim 114, further comprising a step of:

c) drying the formulation.

117. A method of preparing a dosage form, the method comprising a step of:

a) diluting a formulation of claim 111;

b) thawing and diluting a formulation of claim 112; and/or

c) resuspending and diluting a formulation of claim 113.

118. The method of claim 117, further comprising administering the dosage form to a subject in need of thereof.

119. The method of claim 118, wherein the subject is in need of an expression product of the mRNA.

120. A method comprising a step of:

administering a dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

~~ii) ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml;~~

~~iii) 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml;~~

~~iv) distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml;~~

~~v) cholesterol at a concentration of about 3.1 mg/ml;~~

~~b) sucrose at a concentration of about 5% w/v in the formulation;~~

~~c) trehalose at a concentration of about 5% w/v in the formulation;~~

~~d) HEPES buffer, wherein the HEPES buffer is substantially free of sodium chloride and is at a concentration of about 10 mM in the formulation;~~

~~wherein the formulation is diluted into the dosage form prior to administration.~~

~~121~~11. A formulation comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) sucrose at a concentration of about 10% w/v ~~in the formulation~~; and

c) PBS buffer, wherein the PBS buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride.

~~122. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide~~

~~(ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 10% w/v in the formulation;~~

~~c) PBS buffer, wherein the PBS buffer is substantially free of sodium chloride.~~

~~123. A dry formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 10% w/v in the formulation before drying;~~

~~c) PBS buffer, wherein the PBS buffer is substantially free of sodium chloride.~~

~~124~~12. A method of preparing a formulation of claim 11, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein the PBS buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride; and

ii) sucrose at a concentration of about 10% w/v ~~in the formulation~~.

~~125. The method of claim 124, further comprising a step of:~~

~~c) freezing the formulation.~~

~~126. The method of claim 124, further comprising a step of:~~

~~c) drying the formulation.~~

~~127. A method of preparing a dosage form, the method comprising a step of:~~

~~a) diluting a formulation of claim 121;~~

~~b) thawing and diluting a formulation of claim 122; and/or~~

~~c) resuspending and diluting a formulation of claim 123.~~

~~128. The method of claim 127, further comprising administering the dosage form to a subject in need of thereof.~~

~~129. The method of claim 128, wherein the subject is in need of an expression product of the mRNA.~~

~~130~~13. ~~A method comprising a step of:~~

~~administering a~~A dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) a neutral lipid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml~~;

iii) a polymer-conjugaed lipid~~2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml~~;

iv) a cationic lipid~~distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml~~;

v) a steroid~~cholesterol at a concentration of about 3.1 mg/ml~~;

b) sucrose at a concentration of about 10% w/v; and

c) PBS buffer, wherein the PBS buffer is i) substantially free of sodium chloride, or ii) comprises about 6 mg/ml sodium chloride;

wherein the dosage form is dilution from the formulation ~~is diluted into the dosage form prior to administration~~.

~~1312~~14. ~~A~~ The formulation of claim 11, comprising:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) sucrose at a concentration of about 10% w/v ~~in the formulation~~;

c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride ~~in the formulation~~.

~~132. A frozen formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 10% w/v in the formulation;~~

~~c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride in the formulation.~~

~~133. A dry formulation comprising:~~

~~a) a lipid nanoparticle (LNP), wherein the LNP comprises:~~

~~i) a payload that is or comprises one or more mRNAs;~~

~~ii) lipids that include: ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5; and~~

~~b) sucrose at a concentration of about 10% w/v in the formulation before drying;~~

~~c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride in the formulation before drying.~~

~~134~~15. A method of preparing a formulation of claim 14, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid ~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride ~~in the formulation~~; and

ii) sucrose at a concentration of about 10% w/v ~~in the formulation~~.

~~135. The method of claim 134, further comprising a step of:~~

~~c) freezing the formulation.~~

~~136. The method of claim 134, further comprising a step of:~~

~~c) drying the formulation.~~

~~137. A method of preparing a dosage form, the method comprising a step of:~~

~~a) diluting a formulation of claim 131;~~

~~b) thawing and diluting a formulation of claim 132; and/or~~

~~c) resuspending and diluting a formulation of claim 133.~~

~~138. The method of claim 137, further comprising administering the dosage form to a subject in need of thereof.~~

~~139. The method of claim 138, wherein the subject is in need of an expression product of the mRNA.~~

~~140~~16. ~~A method comprising a step of:~~

~~administering a~~A dosage form of a formulation, wherein the formulation comprises:

a) a lipid nanoparticle (LNP), wherein the LNP comprises:

i) mRNA at a concentration of about 0.5 mg/ml;

ii) a neutral lipid~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315) at a concentration of about 7.17 mg/ml~~;

iii) a polymer-conjugated lipid~~2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159) at a concentration of about 0.89 mg/ml~~;

iv) a cationic lipid~~distearoylphosphatidylcholine (DSPC) at a concentration of about 1.56 mg/ml~~;

v) a steroid~~cholesterol at a concentration of about 3.1 mg/ml~~;

b) sucrose at a concentration of about 10% w/v ~~in the formulation~~; and

c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride;

wherein the dosage form is diluted into the formulation ~~is diluted into the dosage form prior to administration~~.

~~141~~17. A method of preparing a formulation of claim 14, the method comprising steps of:

a) preparing a lipid nanoparticle (LNP) in a first buffer system, wherein the LNP comprises:

i) a payload that ~~is or~~ comprises one or more mRNAs;

ii) lipids that include: a cationic lipid, a neutral lipid, a polymer conjugated lipid, and a steroid ~~((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate) (ALC-0315); 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (ALC-0159); distearoylphosphatidylcholine (DSPC); and cholesterol at relative mass ratios in a range of about 8:1:1.5:3 to about 9:1:2:3.5~~; and

b) exchanging the first buffer system for a second buffer system, wherein the second buffer system comprises:

i) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride ~~in the formulation~~; and

ii) sucrose at a concentration of about 10% w/v ~~in the formulation~~

wherein the first buffer system comprises sucrose at a concentration of about 10% w/v.

~~142. The method of claim 141, further comprising a step of:~~

~~c) freezing the formulation.~~

~~143. The method of claim 141, further comprising a step of:~~

~~c) drying the formulation.~~

~~144~~18. A formulation of any one of claims 1, 2, 5, 8, 11, or 14 or a dosage form according to any one of claims 4, 7, 10, 13 or 16 for use in ~~method of~~ delivering a nucleic acid into a cell in a subject ~~comprising a step of administering a formulation as described in any of the preceding claims~~.

~~145~~19. A formulation of any one of claims 1, 2, 5, 8, 11, or 14 or a dosage form according to any one of claims 4, 7, 10, 13 or 16 for use in ~~method of~~ inducing an immune response in a subject ~~comprising a step of administering to the subject a formulation as described in any of the preceding claims~~.

~~146~~20. The ~~method~~ formulation or dosage form for use of claim ~~145~~19, wherein the immune response is against a viral antigen, or epitope thereof, encoded by the mRNA.

~~147~~21. The formulation or dosage form for use ~~method~~ of claim ~~146~~20, wherein the viral antigen is an antigen of a coronavirus.

~~148~~22. The formulation or dosage form for use ~~method~~ of claim ~~147~~21, wherein the coronavirus is a SARS-CoV2 virus.

~~149~~23. The formulation or dosage form for use ~~method~~ of claim ~~147~~ 21 or claim ~~148~~22, wherein the antigen ~~is or~~ comprises an S protein.

~~150. The formulation of any one of claims 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, or 133, or the method of any one of claims 6, 16, 26, 36, 46, 56, 66, 76, 86, 96, 106, 116, 126, 136, or 143, wherein the formulation is dried until it is substantially free of water.~~

~~151. The formulation of any one of claims 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, 133, or 150, or the method of any one of claims 6, 16, 26, 36, 46, 56, 66, 76, 86, 96, 106, 116, 126, 136, 143, or 150, wherein the formulation is dried by lyophilization.~~

~~152. The formulation of any one of claims 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, 133, 150, or 151, or the method of any one of claims 6, 16, 26, 36, 46, 56, 66, 76, 86, 96, 106, 116, 126, 136, 143, 150, or 151, wherein the formulation is dried until it comprises less than 0.8%, less than 0.7%, less than 0.6%, less than 0.5%, less than 0.4%, or less than 0.3% w/w water.~~

~~153. The formulation of any one of claims 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, 133, 150, 151, or 152, or the method of any one of claims 6, 16, 26, 36, 46, 56, 66, 76, 86, 96, 106, 116, 126, 136, 143, 150, 151, or 152, wherein the formulation is annealed during drying.~~

~~154. The formulation of any one of claims 3, 13, 23, 33, 43, 53, 63, 73, 83, 93, 103, 113, 123, 133, 150, 151, or 152, or the method of any one of claims 6, 16, 26, 36, 46, 56, 66, 76, 86, 96, 106, 116, 126, 136, 143, 150, 151, or 152, wherein the formulation is not annealed during drying.~~

~~155. The formulation of any one of claims 150-154, or the method of any one of claims 150-154, wherein the formulation maintains less than 0.8%, less than 0.7%, less than 0.6%, less than 0.5%, less than 0.4%, or less than 0.3% w/w water for at least 12 weeks at temperatures ranging from about 2ºC to about 25ºC.~~

~~156~~24. The formulation of any one of claims 1-2, 5, 8, 11, or 14~~1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133~~, ~~or~~ the method of any one of claims 3, 6, 9, 12, 15, or 17, the dosage form of any one of claims 4, 7, 10, 13 or 16, or the formulation or dosage form for use of any one of claims 18 to 23~~4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149~~, wherein the one or more mRNAs encodes one or more polypeptides.

~~157~~25. The formulation, method, dosage form or the formulation or dosage form for use of claim ~~156~~24, wherein the one or more polypeptides ~~are or~~ comprise an epitope for inducing an immune response against an antigen in a subject.

~~158~~26. The formulation of any one of claims 1-2, 5, 8, 11, or 14~~1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133~~, wherein the one or more mRNAs ~~are or~~ comprise an epitope for inducing an immune response against an antigen in a subject.

~~159~~27. The formulation of any one of claims 1-2, 5, 8, 11, 14, or 26~~1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, 131-133, or 158~~, or the method of any one of claims 3, 6, 9, 12, 15, or 17, the dosage form of any one of claims 4, 7, 10, 13 or 16, or the formulation or dosage form for use of any one of claims 18 to 23~~4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149~~, wherein the one or more RNAs ~~are or~~ comprise self-amplifying RNA molecules.

~~160~~28. The formulation of any one of claims 1-2, 5, 8, 11, 14, 26 or 27~~1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, 131-133, 158, or 159~~, or the method of any one of claims 3, 6, 9, 12, 15, 17 or 27, the dosage form of any one of claims 4,

7, 10, 13, 16 or 27, or the formulation or dosage form for use of any one of claims 18 to 23 or 274-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, 141-149, or 159, wherein the one or more RNAs are or comprise modified RNA molecules, or non-modified RNA molecules.

16129. The formulation of any one of claims 1-2, 5, 8, 11, 14 or 26-281-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, 131-133, or 158-160, or the method of any one of claims 3, 6, 9, 12, 15, 17, 27 or 28, the dosage form of any one of claims 4, 7, 10, 13, 16, 27 or 28, or the formulation or dosage form for use of any one of claims 18 to 23, 27 or 284-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, 141-149, 159, or 160, wherein the one or more RNAs are or comprise non-modified uridine RNA molecules.

16230. The formulation of any one of claims 11-2, 5, 8, 11, 14, or 26-29-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, 131-133, or 158-161, or the method of any one of claims 3, 6, 9, 12, 15, 17, 27 to 29, the dosage form of any one of claims 4, 7, 10, 13, 16, 27 to 29, or the formulation or dosage form for use of any one of claims 18 to 23, 27 to 294-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, 141-149, or 159-161, wherein the one or more RNAs are or comprise nucleoside modified RNA molecules.

16331. The formulation, method, dosage form or the formulation or dosage form for use of claim 15624, or the method of claim 156, wherein at least one of the one or more polypeptides is derived from a SARS-COV-2 S Protein of SEQ ID NO: 1 or 7.

16432. The formulation, method, dosage form or the formulation or dosage form for use of claim 15624, or the method of claim 156, wherein at least one of the one or more polypeptides comprises at least 85% sequence identity to the SARS-COV-2 S Protein of SEQ ID NO: 1 or 7.

~~165~~33. The formulation, method, dosage form or the formulation or dosage form for use of claim ~~156~~24~~, or the method of claim 156~~, wherein at least one of the one or more polypeptides is or comprises a full length SARS-COV-2 S Protein of SEQ ID NO: 1 or 7.

~~166~~34. The formulation, method, dosage form or the formulation or dosage form for use of claim ~~156~~24~~, or the method of claim 156~~, wherein at least one of the one or more polypeptides comprises at least 85% sequence identity to the Receptor Binding Domain (RBD) of SARS-COV-2 S Protein of SEQ ID NO: 1 or 7.

~~167~~35. The formulation, method, dosage form or the formulation or dosage form for use of claim ~~156~~24~~, or the method of claim 156~~, wherein at least one of the one or more polypeptides ~~is or~~ comprises a Receptor Binding Domain (RBD) of SARS-COV-2 S Protein of SEQ ID NO: 1 or 7.

~~168~~36. The formulation of any one of claims 1-2, 5, 8, 11, or 14~~1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133~~, or the method of any one of claims 3, 6, 9, 12, 15, or 17, the dosage form of any one of claims 4, 7, 10, 13 or 16, or the formulation or dosage form for use of any one of claims 18 to 23~~4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149~~, wherein the one or more mRNAs are associated or encapsulated in the LNP.

37. The formulation of any one of claims 5, 8, 11, or 14, wherein the formulation is a frozen formulation.

38. A dose of the formulation of any one of claims 1-2, 5, 8, 11, 14, 24-36, or 37, which dose includes 1 µg to 100 µg of mRNA.

39. A dose of the formulation of claims 1-2, 5, 8, 11, 14, 24-36, or 37, which dose includes less than 60 µg of mRNA.

40. The method of any one of claims 3, 6, 9, 12, 15, 17, 24, 25 or 27-36, wherein the formulation includes 1 µg to 100 µg of mRNA per dose.

41. The method of any one of claims 3, 6, 9, 12, 15, 17, 24, 25 or 27-36, wherein the formulation includes less than 60 µg of mRNA per dose.

42. The dosage form of any one of claims 4, 7, 10, 13, 16, 24, 25 or 27-36, or the dosage form for use of any one of claims 18-23, wherein 1 µg to 100 µg of mRNA is to be administered.

43. The dosage form of any one of claims 4, 7, 10, 13, 16, 24, 25 or 27-36, or the dosage form for use of any one of claims 18-23, wherein less than 60 µg of mRNA is to be administered.

~~169. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation has been stored over a time period of at least 12 weeks at a temperature above 25ºC.~~

~~170. The of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation has been stored over a time period of at least 12 weeks at a temperature in a range of about 2-40ºC.~~

~~171. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation is characterized in that lipid nanoparticles exhibit less than 20 nm change in Z-Average when stored over a time period of at least 12 weeks at a temperature of about 2-8ºC as compared to a control formulation.~~

~~172. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-~~

~~139, or 141-149, wherein the formulation is characterized in that lipid nanoparticles exhibit less than 20 nm change in Z-Average when stored over a time period of at least 12 weeks at a temperature of about 25ºC as compared to a control formulation.~~

~~173. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation is characterized in that lipid nanoparticles exhibit less than 0.1 change in Polydispersity Index (PDI) when stored over a time period of at least 12 weeks at a temperature of about 2-8ºC as compared to a control formulation.~~

~~174. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation is characterized in that lipid nanoparticles exhibit less than 0.1 change in Polydispersity Index (PDI) when stored over a time period of at least 12 weeks at a temperature of about 25ºC as compared to a control formulation.~~

~~175. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation is characterized by a %mRNA encapsulation of at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% when stored over a time period of at least 12 weeks at a temperature of about 2-8ºC as compared to a control formulation.~~

~~176. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation is characterized by mRNA encapsulation% of at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% when stored over a~~

~~time period of at least 12 weeks at a temperature of about 25ºC as compared to a control formulation.~~

~~177. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation is characterized by a %mRNA expression of at least 60%, at least 70%, at least 80%, or at least 90% when stored over a time period of at least 12 weeks at a temperature of about 2-8ºC as compared to a control formulation.~~

~~178. The formulation of any one of claims 1-3, 11-13, 21-23, 31-33, 41-43, 51-53, 61-63, 71-73, 81-83, 91-93, 101-103, 111-113, 121-123, or 131-133, or the method of any one of claims 4-9, 14-19, 24-29, 34-39, 44-49, 54-59, 64-69, 74-79, 84-89, 94-99, 104-109, 114-119, 124-129, 134-139, or 141-149, wherein the formulation is characterized by a %mRNA expression of at least 60%, at least 70%, at least 80%, or at least 90% when stored over a time period of at least 12 weeks at a temperature of about 25ºC as compared to a control formulation.~~