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 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 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 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 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 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 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 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 examples, methods of providing such formulations described herein are also described herein. In some 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 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 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 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.

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

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 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.

4. 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.

5. The formulation of claim 1, 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) Tris buffer, wherein the Tris buffer is substantially free of sodium chloride and is at a concentration of about 10 mM.

6. The method of 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 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 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.

7. The dosage form of claim 4, 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) Tris buffer, wherein the Tris 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.

8. 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.

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 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.

10. 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.

11. 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.

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 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.

13. 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.

14. The formulation of claim 11, 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;

c) PBS buffer, wherein the PBS buffer comprises about 6 mg/ml sodium chloride.

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 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.

16. 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.

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 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.

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 delivering a nucleic acid into a cell in a subject.

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 inducing an immune response in a subject.

20. The formulation or dosage form for use of claim 19, wherein the immune response is against a viral antigen, or epitope thereof, encoded by the mRNA.

21. The formulation or dosage form for use of claim 20, wherein the viral antigen is an antigen of a coronavirus.

22. The formulation or dosage form for use of claim 21, wherein the coronavirus is a SARS-CoV2 virus.

23. The formulation or dosage form for use of claim 21 or claim 22, wherein the antigen comprises an S protein.

24. The formulation of any one of claims 1-2, 5, 8, 11, or 14, 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, wherein the one or more mRNAs encodes one or more polypeptides.

25. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, wherein the one or more polypeptides comprise an epitope for inducing an immune response against an antigen in a subject.

26. The formulation of any one of claims 1-2, 5, 8, 11, or 14, wherein the one or more mRNAs comprise an epitope for inducing an immune response against an antigen in a subject.

27. The formulation of any one of claims 1-2, 5, 8, 11, 14, or 26, 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, wherein the one or more RNAs comprise self-amplifying RNA molecules.

28. The formulation of any one of claims 1-2, 5, 8, 11, 14, 26 or 27, 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 27, wherein the one or more RNAs comprise modified RNA molecules, or non-modified RNA molecules.

29. The formulation of any one of claims 1-2, 5, 8, 11, 14 or 26-28, 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 28, wherein the one or more RNAs comprise non-modified uridine RNA molecules.

30. The formulation of any one of claims 1-2, 5, 8, 11, 14, or 26-29, 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 29, wherein the one or more RNAs comprise nucleoside modified RNA molecules.

31. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, 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.

32. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, 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.

33. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, 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.

34. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, 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.

35. The formulation, method, dosage form or the formulation or dosage form for use of claim 24, wherein at least one of the one or more polypeptides comprises a Receptor Binding Domain (RBD) of SARS-COV-2 S Protein of SEQ ID NO: 1 or 7.

36. The formulation of any one of claims 1-2, 5, 8, 11, or 14, 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, 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.