DRY POWDER FORMULATIONS FOR MESSENGER RNA

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 62/702,193 filed July 23, 2018; incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on July 19, 2019, is named MRT_2008WO_SeqListing.txt and is 1137 byte in size.

BACKGROUND

[0003] Messenger RNA therapy (MRT) is becoming an increasingly important approach for the treatment of a variety of diseases. Lipid encapsulated mRNA formulations, such as lipid nanoparticle (LNP) compositions show high degree of cellular uptake and protein expression. However, presently these formulations are typically in liquid forms, and are required to be administered usually in the form of injections, or via nebulizers. These modes of administration are less desired by the patient than some less invasive routes, for example, metered dose inhalers. Lyophilized formulations sometimes do not offer reliable particle uniformity in dry state, or ease of handling and distribution. Lyophilized powder has to be dissolved in an appropriate solvent prior to dispensing to a patient and can undergo degradation within a few hours. Repeated freeze thawing of mRNA preparations is not recommended due to the potential for mRNA and/or LNP instability.

SUMMARY

[0004] The present disclosure provides, among other things, a dry powder (i.e., spray-dried) formulation of mRNA encapsulated with lipid based nanoparticles for more efficient mRNA delivery and more efficacious mRNA therapy. Prior to the present disclosure, one of the challenges of spray-drying lipid nanoparticle-encapsulated mRNA arose from the fact that both mRNA and the lipid nanoparticle components are structurally labile at the high temperatures and/or pressures needed for adequate spray-drying. For example, an inlet

temperature of a spray-dryer ranges between 80ºC to 98ºC. Lipids tend to melt and/or aggregate at the high inlet temperature at or near the spray nozzle. This causes hindrance to the flow of the formulation through the nozzle into the drying chamber, disrupts uniform dispersion of the spray and produces undesirable particle characteristics and poor yield. The present disclosure has unexpectedly solved this problem with the addition of a polymer to the mRNA and lipid nanoparticle mixture before subjecting the mixture to the spray-drying process. As described herein, the inventors observed that adding a polymer to an mRNA and lipid mixture effectively prevents aggregation of lipid nanoparticles and facilitates dry powder formation of fine particles containing mRNA-loaded lipid nanoparticles suitable for inhalation.

[0005] More surprisingly, despite the extremely unstable nature of mRNA, dry powder formulations prepared according to the present disclosure, even under high temperatures and/or high pressures associated with spray-drying, are stable and able to maintain a high degree of mRNA integrity even after long term storage at various temperatures. In addition, a dry powder formulation prepared according to the present disclosure is also characterized with high LNP encapsulation efficiency of mRNA, resulting in high cellular delivery of mRNA. Therefore, the present disclosure fulfils a long-standing need in the mRNA therapy field for a stable dry powder form of mRNA therapeutic, which can easily be stored, transferred, and dispensed. Further, the dry powder formulations of mRNA according to the present disclosure can be administered as dry powder to a patient, e.g. in metered doses or weighed out and reconstituted in single-use amounts, without the need for freezing single-use aliquots of liquid.

[0006] In one aspect, the present disclosure provides a dry powder formulation for delivery of messenger RNA (mRNA) containing a plurality of spray-dried particles comprising mRNA encoding a protein or a peptide, one or more lipids, and one or more polymers.

[0007] In another aspect, the present disclosure provides a dry powder formulation for delivery of messenger RNA (mRNA) containing a plurality of spray-dried particles comprising one or more lipid nanoparticles (LNPs) encapsulating mRNA encoding a peptide or polypeptide, and one or more polymers.

[0008] In still another aspect, the present disclosure provides a dry powder formulation for delivery of messenger RNA (mRNA) containing a plurality of spray-dried

particles comprising one or more nanoparticles encapsulating mRNA encoding a peptide or polypeptide, the nanoparticles comprising one or more lipids, and one or more polymers.

[0009] In yet another aspect, the present disclosure provides a dry powder formulation for delivery of cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) containing a plurality of spray-dried particles comprising mRNA encoding a CFTR protein, one or more lipids, and one or more polymers. In some examples, the one or more lipids form one or more lipid nanoparticles (LNPs) encapsulating the mRNA encoding the CFTR protein. In some examples, the one or more lipids and the one or more polymers form one or more nanoparticles encapsulating the mRNA encoding the CFTR protein.

[0009a] In another aspect, the present disclosure provides use of the dry powder formulation as described herein in the manufacture of a pharmaceutical composition for delivering cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) for *in vivo* expression, comprising that the dry powder formulation is to be administered to a subject in need thereof. In another aspect, the present disclosure provides use of the dry powder formulation as described herein in the manufacture of a pharmaceutical composition for delivering cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) for *in vivo* expression, wherein the use comprises reconstituting the dry powder formulation into a liquid solution; and wherein the reconstituted liquid solution is to be administered to a subject in need thereof. In yet another aspect, the present disclosure provides use of the dry powder formulation as described herein in the manufacture of a pharmaceutical composition for delivering mRNA *in vivo* to a subject in need thereof. In yet another aspect, the present disclosure provides use of the dry powder formulation as described herein in the manufacture of a pharmaceutical composition for treating a disease or disorder in a patient, comprising that an effective dose of mRNA in the dry powder formulation is to be administered to the patient.

[0009b] In a further aspect, the present disclosure provides a dry power formulation manufactured according to a method as described herein. In a further aspect, the present disclosure provides use of the dry powder formulation as described herein in the manufacture of a pharmaceutical composition for treating a disease or disorder in a patient, comprising that an effective dose of mRNA in the dry powder formulation is to be administered to the patient.

[0010] As used in this application, lipid nanoparticles (LNPs) encompass nanoparticles formed with lipids, as well as nanoparticles formed with both lipids and polymers. In some examples, nanoparticles formed with both lipids and polymers are referred to as lipid-polymer nanoparticles.

[0011] In some examples, mRNA (e.g., CFTR mRNA) has an integrity of 80% or greater, 85% or greater, 90% or greater, 95% or greater, 96% or greater, 97% or greater, 98% or greater, or 99% or greater. In some examples, mRNA (e.g., CFTR mRNA) has an integrity of 90% or greater. In some examples, mRNA (e.g., CFTR mRNA) has an integrity of 95% or greater. In some examples, mRNA (e.g., CFTR mRNA) has an integrity of 98% or greater.

[0012] In some examples, the mRNA maintains an integrity of 90% or greater upon storage at or under room temperature for 6 months or longer. In some examples, the mRNA maintains an integrity of 95% or greater upon storage at or under room temperature for 6 months or longer. In some examples, the mRNA maintains an integrity of 98% or greater upon storage at or under room temperature for 6 months or longer.

[0013] In some examples, the mRNA maintains integrity of or greater than 90% after spray-drying and storage at or under room temperature for three months or longer. In some examples, the mRNA maintains integrity of or greater than 90% after spray-drying and storage at or under room temperature for six months or longer. In some examples, the mRNA maintains integrity of or greater than 90% after spray-drying and storage at or under room temperature for nine months or longer. In some examples, the mRNA maintains integrity of or greater than 90% after spray-drying and storage at or under room temperature for twelve months or longer. In some examples, the mRNA maintains integrity of or greater than 90% after spray-drying and storage at or under 4°C for three months or longer. In some examples, the mRNA maintains integrity of or greater than 90% after spray-drying and storage at or under 4°C for six months or longer. In some examples, the mRNA maintains integrity of or greater than 90% after spray-drying and storage at or under 4°C or lower for nine months or longer. In some examples, the mRNA maintains integrity of or greater than 90% after spray-drying and storage at or under 4°C for twelve months or longer. In some examples, the mRNA maintains integrity of or greater than 95% after storage at or under room temperature for 3 months or longer, 6 months or longer, 9 months or longer, or 12 months or longer. In some examples, the mRNA maintains integrity of or greater than 95% after storage at or

under 4°C for 3 months or longer, 6 months or longer, 9 months or longer, or 12 months or longer.

[0014] In some examples, at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50% of the plurality of spray-dried particles are a fraction of fine particles. In some examples, at least 20% of the plurality of spray-dried particles are a fraction of fine particles.

[0015] In some examples, the fine particles have a volume median diameter of 5 micrometers or less. In some examples, the fine particles have a volume median diameter of 4 micrometers or less. In some examples, the fine particles have a volume median diameter of 3 micrometers or less. In some examples, the fine particles have a volume median diameter of 2 micrometers or less. In some examples, the fine particles have a volume median diameter of 1 micrometer or less.

[0016] In some examples, the plurality of spray-dried particles has an average sphericity of greater than 0.6, greater than 0.7, greater than 0.8, or greater than 0.9. In some examples, the plurality of spray-dried particles has a Z-average size of less than 3,000 nm, 2,500 nm, 2,000 nm, 1,500 nm, 1,000 nm, or 500 nm.

[0017] In some examples, the plurality of spray-dried particles comprise a residual moisture content of less than 20%, less than 18%, less than 16%, less than 14%, less than 12%, less than 10%, less than 9%, less than 8%, less than 7%, less than 6%, less than 5%, less than 4%, less than 3%, less than 2%, less than 1%, less than 0.9%, less than 0.8%, less than 0.7%, less than 0.6%, less than 0.5%, less than 0.4%, less than 0.3%, less than 0.2%, or less than 0.1%.

[0018] In some examples, the dry powder formulation is inhalable. In some examples, the dry-powder formulation is inhaled as a dry powder in a metered dose inhaler. In some examples, the dry-power formulation is reconstituted with a diluent and administered by nebulization.

[0019] In some examples, the one or more polymers constitute at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% of the combined weight of the lipids and polymers. In some examples, the one or more polymers constitute about 10-90%, 10-80%, 10-70%, 10-60%, 10-50%, 10-40%, 10-30%, 10-20%, 15-20%, 15-25%, 15-30%, 15-35%, 15-40%, 15-45%, 15-50%, 15-55%, 15-60%, 15-65%, 15-70%, 15-75%, 15-80%, or 15-90% of the combined weight of the lipids and polymers. In some examples, the one or more polymers constitute no more than 90%,

80%, 70%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, or 20% of the combined weight of the lipids and polymers.

[0020] In some examples, the one or more polymers constitute at least 50% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 40% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 30% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 20% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 15% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 12% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 10% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 9% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 8% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 7% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 6% of the total weight of dry powder. In some examples, the one or more polymers constitute at least 5% of the total weight of dry powder.

[0021] In some examples, the one or more polymers are selected from the group consisting of chitosan, poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(q-caprolactone (PCL), poly amido amines, polyesters, polycarbonates, poly(hydroxyalkyl L-asparagine), poly(hydroxyalkyl L-glutamine), poly(2-alkyloxazoline) acrylates, modified acrylates and polymethacrylate based polymers, poly-N-(2-hydroxyl-propyl)methacrylamide, poly-2-(methacryloyloxy)ethyl phosphorylcholines, poly(2- (methacryloyloxy)ethyl phosphorylcholine), and poly(dimethylaminoethyl methylacrylate) (pDMAEMA).

[0022] In some examples, the one or more polymers include a polymethacrylate based polymer. In some examples, the one or more polymers include Eudragit EPO.

[0023] In some examples, the one or more LNPs encapsulating mRNA (also referred to as mRNA-loaded LNPs) have a lipid: mRNA (N/P) ratio ranging from 1-20, 1-15, 1-10, 2-8, 2-6, or 2-4. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 1 to 20. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 1 to 18. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 1 to 16. In some examples, the one or more mRNA-loaded lipid nanoparticles

have a lipid: mRNA (N/P) ratio ranging from 1 to 14. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 1 to 12. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 1 to 10. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 1 to 8. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 1 to 6. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 2 to 20. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 2 to 16. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 2 to 12. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 2 to 8. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 2 to 6. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 2 to 4. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 4 to 20. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 4 to 16. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 4 to 14. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 4 to 12. In some examples, the one or more mRNA-loaded lipid nanoparticles have a lipid: mRNA (N/P) ratio ranging from 4 to 10. In some examples, the one or more mRNA-loaded LNPs have a lipid: mRNA (N/P) ratio of 2 or 4. In some examples, the one or more mRNA-loaded LNPs have a lipid: mRNA (N/P) ratio of 2. In some examples, the one or more mRNA-loaded LNPs have a lipid: mRNA (N/P) ratio of 4.

[0024] In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 70% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 75% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 80% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 85% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 90% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an

encapsulation efficiency of 92% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 94% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 95% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 96% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 97% or greater. In some examples, the one or more mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 98% or greater.

[0025] In some examples, the one or more lipids comprise a cationic lipid. In some examples, the cationic lipid is selected from the group consisting of C12-200, DOTAP (1,2-dioleyl-3-trimethytammonium propane), DODAP (1 ,2-dioleyl-3-dimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DLinDMA, DLin-KC2-DMA, HGT4003, cKK-E12, ICE, and combinations thereof.

[0026] In some examples, the one or more mRNA-loaded lipid nanoparticles comprise one or more cationic lipids. In some examples, the one or more cationic lipids comprises an ionizable cationic lipid. In some examples, the one or more cationic lipids comprises the cationic lipid C12-200. In some examples, the one or more cationic lipids comprises the cationic lipid DOTAP (1,2-dioleyl-3-trimethytammonium propane). In some examples, the one or more cationic lipids comprises the cationic lipid DODAP (1 ,2-dioleyl-3-dimethylammonium propane). In some examples, the one or more cationic lipids comprises the cationic lipid DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane). In some examples, the one or more cationic lipids comprises the cationic lipid DLinDMA. In some examples, the one or more cationic lipids comprises the cationic lipid DLin-KC2-DMA. In some examples, the one or more cationic lipids comprises the cationic lipid HGT-5000. In some examples, the one or more cationic lipids comprises the cationic lipid HGT-5001. In some examples, the one or more cationic lipids comprises the cationic lipid HGT-5002. In some examples, the one or more cationic lipids comprises the cationic lipid cKK-E12. In some examples, the one or more cationic lipids comprises the cationic lipid OF-02. In some examples, the one or more cationic lipids comprises the cationic lipid Target 23. In some examples, the one or more cationic lipids comprises the cationic lipid Compound 1. In some examples, the one or more cationic lipids comprises the cationic lipid Compound 2. In some examples, the one or more cationic lipids comprises the cationic lipid Compound 3. In some

examples, the one or more cationic lipids comprises the cationic lipid HGT4001. In some examples, the one or more cationic lipids comprises the cationic lipid HGT4002. In some examples, the one or more cationic lipids comprises the cationic lipid HGT4003. In some examples, the one or more cationic lipids comprises the cationic lipid HGT4004. In some examples, the one or more cationic lipids comprises the cationic lipid HGT4005. In some examples, the one or more cationic lipids comprises the cationic lipid 18:1 Carbon tail-ribose lipid. In some examples, the one or more cationic lipids comprises the cationic lipid ICE.

[0027] In some examples, the cationic lipid constitutes about 25-50% of the total lipids in LNPs by molar.

[0028] In some examples, the one or more lipids comprise a PEG-modified lipid. In some examples, the one or more mRNA-loaded lipid nanoparticles comprise one or more PEG-modified lipids. In some examples, the one or more PEG-modified lipids comprise a poly(ethylene) glycol chain of up to 5 kDa in length covalently attached to a lipid comprising one or more alkyl chains of C6-C20 in length. In some examples, the one or more PEG-modified lipids constitute up to 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% of the total lipids in LNPs by molar. In some examples, the PEG-modified lipid constitutes about 1-15% of the total lipids in LNPs by molar. In some examples, the PEG-modified lipid constitutes at least 1%, 2%, 3%, 4%, 5%, 6%, 8%, 10%, or 12% of the total lipids in LNPs by molar.

[0029] In some examples, suitable LNPs according to the present disclosure are two-lipid component LNPs.

[0030] In some examples, the one or more lipids do not include a neutral lipid or a cholesterol-based lipid.

[0031] In some examples, the one or more lipids further comprise a neutral lipid and/or a cholesterol-based lipid. In some examples, the one or more lipids further comprise a neutral lipid.

[0032] In some examples, suitable LNPs according to the present disclosure are three-lipid component LNPs.

[0033] In some examples, a dry powder formulation according to the present disclosure further contains at least one sugar. In some examples, the sugar is selected from the group consisting of monosaccharides, disaccharides, polysaccharides, glucose, fructose,

galactose, mannose, sorbose, lactose, sucrose, cellobiose, trehalose, raffinose, starch, dextran, maltodextrin, cyclodextrins, inulin, xylitol, sorbitol, lactitol, mannitol, and combination thereof. In some examples, the sugar is mannitol. In some examples, the sugar constitutes less than 30%, 25%, 20%, 15%, 10%, or 5% of total weight.

[0034] In some examples, a dry powder formulation according to the present disclosure further comprises a pharmaceutically acceptable excipient selected from the group consisting of esters, urethanes, phosphoesters, phosphazenes, amino acids, collagen, chitosan, polysaccharides, albumin, surfactants, buffers, salts, and combinations thereof.

[0035] In some examples, a suitable surfactant is selected from the group consisting of CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate), phospholipids, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, sphingomyelins, Octaethylene glycol monododecyl ether, Pentaethylene glycol monododecyl ether, Triton X-100, Cocamide monoethanolamine, Cocamide diethanolamine, Glycerol monostearate, Glycerol monolaurate, Sorbitan moonolaureate, Sorbitan monostearate, Tween 20, Tween 40, Tween 60, Tween 80, Alkyl polyglucosides, and a poloxamer (e.g., poloxamer 407). In some examples, a suitable surfactant is poloxamer.

[0036] In some examples, a dry powder formulation according to the present disclosure further contains a pharmaceutically acceptable excipient. In some examples, a pharmaceutically acceptable excipient is selected from the group consisting of esters, urethanes, phosphoesters, phosphazenes, amino acids, collagen, chitosan, polysaccharides, albumin, surfactants, buffers, salts, and combination thereof.

[0037] In some examples, a dry powder formulation according to the present disclosure contains a surfactant. In some examples, the surfactant is selected from the group consisting of CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate), phospholipids, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, sphingomyelins, Octaethylene glycol monododecyl ether, Pentaethylene glycol monododecyl ether, Triton X-100, Cocamide monoethanolamine, Cocamide diethanolamine, Glycerol monostearate, Glycerol monolaurate, Sorbitan moonolaureate, Sorbitan monostearate, Tween 20, Tween 40, Tween 60, Tween 80, Alkyl polyglucosides, and copolymers. In some examples, the surfactant is a poloxamer. In some examples, the surfactant is poloxamer a triblock copolymer consisting of a central hydrophobic block of polypropylene glycol flanked

by two hydrophilic blocks of polyethylene glycol (PEG). In some examples, the surfactant is poloxamer 407.

[0038] In some examples, the mRNA constitutes up to 10% of the total weight of dry powder. In some examples, the mRNA constitutes up to 9% of the total weight of dry powder. In some examples, the mRNA constitutes up to 8% of the total weight of dry powder. In some examples, the mRNA constitutes up to 7% of the total weight of dry powder. In some examples, the mRNA constitutes up to 6% of the total weight of dry powder. In some examples, the mRNA constitutes up to 5% of the total weight of dry powder. In some examples, the mRNA constitutes up to 4% of the total weight of dry powder. In some examples, the mRNA constitutes up to 3% of the total weight of dry powder. In some examples, the mRNA constitutes up to 2% of the total weight of dry powder. In some examples, the mRNA constitutes 1-10% of the total weight of dry powder. In some examples, the mRNA constitutes 1-6% of the total weight of dry powder. In some examples, the mRNA constitutes 1-5% of the total weight of dry powder. In some examples, the mRNA constitutes 1-4% of the total weight of dry powder. In some examples, the mRNA constitutes 1-3% of the total weight of dry powder. In some examples, the mRNA constitutes 2-10% of the total weight of dry powder. In some examples, the mRNA constitutes 2-9% of the total weight of dry powder. In some examples, the mRNA constitutes 2-8% of the total weight of dry powder. In some examples, the mRNA constitutes 2-7% of the total weight of dry powder. In some examples, the mRNA constitutes 2-6% of the total weight of dry powder. In some examples, the mRNA constitutes 2-5% of the total weight of dry powder. In some examples, the mRNA is unmodified. In some examples, the mRNA contains one or more modified nucleotides.

[0039] In some examples, the mRNA encodes a peptide. In some examples, the mRNA encodes a therapeutic protein. In some examples, the therapeutic protein is CFTR.

[0040] In some examples, the CFTR mRNA constitutes about 1-20%, 1-15%, 1-10%, 1-8%, 1-6%, 1-5%, 5-15%, or 5-10% of the total weight of the spray-dried particles. In some examples, the CFTR mRNA constitutes about 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 12.5%, or 15% of the total weight of the spray-dried particles.

[0041] In another example, the present disclosure provides a method of delivering cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) for *in vivo* expression comprising a step of administering to a subject in need thereof a dry powder formulation described herein. In some examples, a dry powder formulation described herein

is administered by pulmonary delivery. In some examples, a dry powder formulation is administered by inhalation.

[0042] In a further example, the present disclosure provides a method of delivering cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) for *in vivo* expression comprising steps of: reconstituting a dry powder formulation of described herein into a liquid solution; and administering to a subject in need thereof the reconstituted liquid solution. In some examples, the reconstituted liquid solution is administered by nebulization. In some examples, the subject is suffering from cystic fibrosis.

[0043] In another aspect, the present disclosure provides a method for manufacturing a dry powder formulation including providing a mixture comprising an mRNA, one or more lipids and a polymer; and spray-drying the mixture to form a plurality of particles.

[0044] In some examples, the one or more lipids are first mixed with the mRNA to form mRNA-loaded lipid nanoparticles before adding the polymer.

[0045] In some examples, a method according to the disclosure further includes adding to the mixture one or more excipients prior to spray drying.

[0046] In some examples, the plurality of spray dried particles is characterized by one or more of the following: a) less than 10% moisture content; b) a fraction of fine particles with a volume median diameter less than 5 micrometers; c) Z- average size range of 10- 3000 nm; d) N/P ratio range from 1 to 20; e) mRNA encapsulation efficiency greater than 80%; and f) mRNA integrity greater than 95%.

[0047] In yet another example, the present disclosure provides a method of delivering mRNA *in vivo* comprising administering to a subject in need thereof a dry powder formulation described herein. In some examples, the dry powder formulation is administered via oral, nasal, tracheal, pulmonary or rectal routes. In some examples, the dry powder formulation is administered by inhalation. In some examples, the dry powder formulation is administered by intranasal spray. In some examples, the formulation is administered by a metered-dose inhaler. In some examples, the formulation is administered by a nebulizer.

[0048] In still another example, the present disclosure provides a method of delivering cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) for *in vivo* expression comprising a step of administering to a subject in need thereof a dry powder formulation described herein.

[0049] In still another example, the present disclosure provides a method of treating a disease or disorder in a patient by administering to the patient an effective dose of mRNA in a dry powder formulation described herein. In some examples, the disease or disorder is selected from cystic fibrosis; asthma; COPD; emphysema; primary ciliary dyskinesia (CILD1) with or without situs inversus, or Kartagener syndrome; pulmonary fibrosis; Birt-Hogg-Dube syndrome; hereditary hemorrhagic telangiectasia; alpha-1 antitrypsin deficiency; Cytochrome b positive granulomatous diseases (CGD, X-lined); Cytochrome b positive granulomatous diseases, autosomal recessive; surfactant deficiency diseases, Pulmonary Surfactant Metabolism Dysfunction 1, Pulmonary Surfactant Metabolism Dysfunction 2, Pulmonary Surfactant Metabolism Dysfunction 3; Respiratory distress syndrome of prematurity; tuberculous tuberculosis, lung viral diseases, including influenza, Respiratory Syncytial Virus (RSV).

[0050] Additional objects and advantages of the disclosure will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the disclosure. The objects and advantages of the disclosure will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[0051] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the disclosure, as claimed.

[0052] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several examples of the disclosure and together with the description, serve to explain the principles of the disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0053] The drawings are for illustration purposes only, not for limitation.

[0054] **Figure 1** shows an exemplary graphical illustration of the spray-drying technique of mRNA formulations.

[0055] **Figure 2** shows the percent recovery of LNP-encapsulated mRNA material following spray-drying, without and with polymer in the formulation.

[0056] **Figure 3** depicts spectrophotometric analysis of an mRNA reference for integrity assessment.

[0057] **Figure 4** depicts exemplary data illustrating integrity of mRNA upon extraction from a lipid nanoparticle.

[0058] **Figure 5** depicts exemplary data illustrating integrity of mRNA encapsulated in a LNP in a formulation with polymer at two weeks following spray-drying and storage at 4ºC.

[0059] **Figure 6** depicts exemplary data illustrating integrity of mRNA encapsulated in a LNP in a formulation with polymer at two weeks following spray-drying and storage at -20ºC.

[0060] **Figure 7** depicts exemplary data that shows no effect of storage temperature on integrity of mRNA encapsulated in a LNP in a formulation with polymer and stored for two weeks after spray-drying.

[0061] **Figure 8** depicts exemplary data illustrating integrity of mRNA encapsulated in a LNP in a formulation with polymer at four weeks following spray-drying and storage at 4ºC.

We claim:

1. A dry powder formulation for delivery of cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) comprising a plurality of spray-dried particles comprising

   mRNA encoding a CFTR protein;

   one or more lipids, and

   one or more polymers.

2. The dry powder formulation of claim 1, wherein the one or more lipids are present in one or more lipid nanoparticles (LNPs) encapsulating the mRNA encoding the CFTR protein.

3. The dry powder formulation of claim 1, wherein the one or more lipids and the one or more polymers are present in one or more lipid nanoparticles (LNPs) encapsulating the mRNA encoding the CFTR protein.

4. The dry powder formulation of any one of the preceding claims, wherein the CFTR mRNA has an integrity of 90% or greater.

5. The dry powder formulation of any one of the preceding claims, wherein the mRNA maintains an integrity of 90% or greater upon storage at or under room temperature for 6 months or longer.

6. The dry powder formulation of any one of the preceding claims, wherein at least 20% of the plurality of spray-dried particles are fine particles.

7. The dry powder formulation of claim 6, wherein the fine particles have a volume median diameter of less than 5 μm.

8. The dry powder formulation of any one of the preceding claims, wherein the dry powder formulation is inhalable.

9. The dry powder formulation of any one of claims 1-7, wherein the formulation is nebulizable upon reconstitution.

10. The dry powder formulation of any one of the preceding claims, wherein the one or more polymers constitute at least 10%, at least 15%, at least 20%, at least 25%, at

least 30%, at least 35%, at least 40%, at least 45%, or at least 50% of the combined weight of the lipids and polymers.

11. The dry powder formulation of any one of the preceding claims, wherein the one or more polymers constitute about 10-90%, 10-80%, 10-70%, 10-60%, 10-50%, 10-40%, 10-30%, 10-20%, 15-20%, 15-25%, 15-30%, 15-35%, 15-40%, 15-45%, 15-50%, 15-55%, 15-60%, 15-65%, 15-70%, 15-75%, 15-80%, or 15-90% of the combined weight of the lipids and polymers.

12. The dry powder formulation of any one of the preceding claims, wherein the one or more polymers constitute no more than 90%, 80%, 70%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, or 20% of the combined weight of the lipids and polymers.

13. The dry powder formulation of any one of the preceding claims, wherein the one or more polymers constitute are selected from a group consisting of chitosan, poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(q-caprolactone (PCL), poly amido amines, polyesters, polycarbonates, poly(hydroxyalkyl L-asparagine), poly(hydroxyalkyl L-glutamine), poly(2-alkyloxazoline) acrylates, modified acrylates and polymethacrylate based polymers, poly-N-(2-hydroxyl-propyl)methacrylamide, poly-2-(methacryloyloxy)ethyl phosphorylcholines, poly(2- (methacryloyloxy)ethyl phosphorylcholine), and poly(dimethylaminoethyl methylacrylate) (pDMAEMA).

14. The dry powder formulation of any one of the preceding claims, wherein the one or more polymers comprise a polymethacrylate based polymer.

15. The dry powder formulation of any one of the preceding claims, wherein the one or more polymers comprise Eudragit EPO.

16. The dry powder formulation of any one of claims 2-15, wherein the one or more LNPs encapsulating mRNA have a lipid: mRNA (N/P) ratio ranging from 1 to 20, 1-15, 1-10, 2-8, 2-6, or 2-4.

17. The dry powder formulation of claim 16, wherein the one or more LNPs encapsulating mRNA have a lipid: mRNA (N/P) ratio of 2 or 4.

18. The dry powder formulation of any one of claims 2-17, wherein the LNPs have an encapsulation efficiency of 80% or greater.

19. The dry powder formulation of any one of the preceding claims, wherein the one or more lipids comprise a cationic lipid.

20. The dry powder formulation of claim 19, wherein the cationic lipid is selected from the group consisting of C12-200, DOTAP (1,2-dioleyl-3-trimethytammonium propane), DODAP (1 ,2-dioleyl-3-dimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DLinDMA, DLin-KC2-DMA, HGT4003, cKK-E12, ICE, and combinations thereof.

21. The dry powder formulation of claim 20, wherein the cationic lipid is cKK-E12.

22. The dry powder formulation of claim 20, wherein the cationic lipid is ICE.

23. The dry powder formulation of any one of claims 19-22, wherein the cationic lipid constitutes about 25-50% of the total lipids in LNPs by molar.

24. The dry powder formulation of any one of the preceding claims, wherein the one or more lipids comprise a PEG-modified lipid.

25. The dry powder formulation of claim 24, wherein the PEG-modified lipid constitutes about 1-15% of the total lipids in LNPs by molar.

26. The dry powder formulation of claim 24, wherein the PEG-modified lipid constitutes at least 1%, 2%, 3%, 4%, 5%, 6%, 8%, 10%, or 12% of the total lipids in LNPs by molar.

27. The dry powder formulation of any one of claims 2-26, wherein the LNPs are two-lipid component LNPs.

28. The dry powder formulation of any one of the preceding claims, wherein the one or more lipids do not comprise a neutral lipid or a cholesterol-based lipid.

29. The dry powder formulation of any one of the preceding claims, wherein the one or more lipids further comprise a neutral lipid or a cholesterol-based lipid.

30. The dry powder formulation of any one of the preceding claims, wherein the one or more lipids further comprise a neutral lipid.

31. The dry powder formulation of claim 29 or 30, wherein the LNPs are three-lipid component LNPs.

32. The dry powder formulation of any one of the preceding claims, further comprising at least one sugar selected from the group consisting of monosaccharides, disaccharides, polysaccharides, glucose, fructose, galactose, mannose, sorbose, lactose, sucrose, cellobiose, trehalose, raffinose, starch, dextran, maltodextrin, cyclodextrins, inulin, xylitol, sorbitol, lactitol, and mannitol.

33. The dry powder formulation of claim 32, wherein the sugar is mannitol.

34. The dry powder formulation of any one of the preceding claims, further comprising a pharmaceutically acceptable excipient selected from the group consisting of esters, urethanes, phosphoesters, phosphazenes, amino acids, collagen, chitosan, polysaccharides, albumin, surfactants, buffers, salts, and combinations thereof.

35. The dry powder formulation of claim 34, wherein the surfactant is selected from the group consisting of CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate), phospholipids, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, sphingomyelins, Octaethylene glycol monododecyl ether, Pentaethylene glycol monododecyl ether, Triton X-100, Cocamide monoethanolamine, Cocamide diethanolamine, Glycerol monostearate, Glycerol monolaurate, Sorbitan moonolaureate, Sorbitan monostearate, Tween 20, Tween 40, Tween 60, Tween 80, Alkyl polyglucosides, and a poloxamer.

36. The dry powder formulation of claim 35, wherein the surfactant is poloxamer.

37. The dry powder formulation of any one of the preceding claims, wherein the CFTR mRNA constitutes about 1-20%, 1-15%, 1-10%, 1-8%, 1-6%, 1-5%, 5-15%, or 5-10% of the total weight of the spray-dried particles.

38. The dry powder formulation of any one of the preceding claims, wherein the CFTR mRNA constitutes about 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 12.5%, or 15% of the total weight of the spray-dried particles.

39. Use of the dry powder formulation of any one of the preceding claims in the manufacture of a pharmaceutical composition for delivering cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) for *in vivo* expression, comprising that the dry powder formulation is to be administered to a subject in need thereof.

40. The use of claim 39, wherein the dry powder formulation is to be administered by pulmonary delivery.

41. The use of claim 39 or 40, wherein the dry powder formulation is to be administered by inhalation.

42. Use of the dry powder formulation of any one of claims 1-38 in the manufacture of a pharmaceutical composition for delivering cystic fibrosis conductance regulator (CFTR) messenger RNA (mRNA) for *in vivo* expression, wherein the use comprises reconstituting the dry powder formulation into a liquid solution; and
wherein the reconstituted liquid solution is to be administered to a subject in need thereof.

43. The use of claim 42, wherein the reconstituted liquid solution is to be administered by nebulization.

44. The use of any one of claims 39-43, wherein the subject is suffering from cystic fibrosis.

45. A dry powder formulation for delivery of messenger RNA (mRNA) comprising a plurality of spray-dried particles comprising

mRNA encoding a protein or a peptide;

one or more lipids, and

one or more polymers.

46. A dry powder formulation for delivery of messenger RNA (mRNA) comprising a plurality of spray-dried particles comprising

a. one or more lipid nanoparticles (LNPs) encapsulating mRNA encoding a peptide or polypeptide, and

b. one or more polymers.

47. A dry powder formulation for delivery of messenger RNA (mRNA) comprising a plurality of spray-dried particles comprising one or more nanoparticles encapsulating mRNA encoding a peptide or polypeptide, the nanoparticles comprising

a. one or more lipids, and

b. one or more polymers.

48. The dry powder formulation of any one of claims 45-47, wherein the mRNA has an integrity of 90% or greater.

49. The dry powder formulation of any one of claims 45-48, wherein the mRNA maintains an integrity of 90% or greater after storage at or under room temperature for 6 months or longer.

50. The dry powder formulation of any one of claims 45-48, wherein the mRNA maintains an integrity of 90% or greater after storage at or under 4°C for 6 months or longer.

51. The dry powder formulation of any one of claims 45-50, wherein at least 20% of the plurality of spray-dried particles are fine particles.

52. The dry powder formulation of claim 51, wherein the fine particles have a volume median diameter of less than 5 μm.

53. The dry powder formulation of any one of claims 45-52, wherein the dry powder formulation is inhalable.

54. The dry powder formulation of any one of claims 45-52, wherein the formulation is nebulizable upon reconstitution.

55. The dry powder formulation of any one of claims 46-54, wherein the one or more LNPs encapsulating mRNA have a lipid: mRNA (N/P) ratio ranging from 1 to 20, 1-15, 1-10, 2-8, 2-6, or 2-4.

56. The dry powder formulation of claim 55, wherein the one or more LNPs encapsulating mRNA have a lipid: mRNA (N/P) ratio of 2 or 4.

57. The dry powder formulation of any one of claims 46-56, wherein the mRNA-loaded lipid nanoparticles have an encapsulation efficiency of 80% or greater.

58. The dry powder formulation of any one of claims 46-57, wherein the one or more mRNA-loaded lipid nanoparticles comprise one or more cationic lipids.

59. The dry powder formulation of claim 58, wherein the one or more cationic lipids are selected from the group consisting of C12-200, DOTAP (1,2-dioleyl-3-trimethytammonium propane), DODAP (1 ,2-dioleyl-3-dimethylammonium propane), DOTMA (1,2-di-O-octadecenyl-3-trimethylammonium propane), DLinDMA, DLin-KC2-DMA, HGT4003, cKK-E12, ICE, and combinations thereof.

60. The dry powder formulation of any one of claims 46-59, wherein the one or more mRNA-loaded lipid nanoparticles comprise one or more PEG-modified lipids.

61. The dry powder formulation of any one of claims 46-60, wherein the LNPs are two-lipid component LNPs.

62. The dry powder formulation of any one of claims 46-60, wherein the one or more mRNA-loaded lipid nanoparticles further comprise one or more neutral lipids or one or more cholesterol-based lipids.

63. The dry powder formulation of claim 62, wherein the LNPs are three-lipid component LNPs.

64. The dry powder formulation of any one of claims 45-63, wherein the one or more polymers constitute less than 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6% or 5% of total weight.

65. The dry powder formulation of any one of claims 45-64, wherein the one or more polymers constitute at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, or at least 50% of the combined weight of the lipids and polymers.

66. The dry powder formulation of any one of claims 45-65, wherein the one or more polymers constitute about 10-90%, 10-80%, 10-70%, 10-60%, 10-50%, 10-40%, 10-30%, 10-20%, 15-20%, 15-25%, 15-30%, 15-35%, 15-40%, 15-45%, 15-50%, 15-55%, 15-60%, 15-65%, 15-70%, 15-75%, 15-80%, or 15-90% of the combined weight of the lipids and polymers.

67. The dry powder formulation of any one of claims 45-66, wherein the one or more polymers constitute no more than 90%, 80%, 70%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, or 20% of the combined weight of the lipids and polymers.

68. The dry powder formulation of any one of claims 45-67, wherein the one or more polymers are selected from a group consisting chitosan, poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(q-caprolactone (PCL), poly amido amines, polyesters, polycarbonates, poly(hydroxyalkyl L-asparagine), poly(hydroxyalkyl L-glutamine), poly(2-alkyloxazoline) acrylates, modified acrylates and polymethacrylate based polymers, poly-N-(2-hydroxyl-propyl)methacrylamide, poly-2-

(methacryloyloxy)ethyl phosphorylcholines, poly(2- (methacryloyloxy)ethyl phosphorylcholine), and poly(dimethylaminoethyl methylacrylate) (pDMAEMA).

69. The dry powder formulation of any one of claims 45-68, wherein the one or more polymers comprise a polymethacrylate based polymer.

70. The dry powder formulation of any one of claims 45-69, further comprising at least one sugar selected from the group consisting of monosaccharides, disaccharides, polysaccharides, glucose, fructose, galactose, mannose, sorbose, lactose, sucrose, cellobiose, trehalose, raffinose, starch, dextran, maltodextrin, cyclodextrins, inulin, xylitol, sorbitol, lactitol, and mannitol.

71. The dry powder formulation of claim 70, wherein the sugar is mannitol.

72. The dry powder formulation of any one of claims 45-71, further comprising a pharmaceutically acceptable excipient selected from the group consisting of esters, urethanes, phosphoesters, phosphazenes, amino acids, collagen, chitosan, polysaccharides, albumin, surfactants, buffers, salts, and combination thereof.

73. The dry powder formulation of claim 72, wherein the surfactant is selected from the group consisting of CHAPS (3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate), phospholipids, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, sphingomyelins, Octaethylene glycol monododecyl ether, Pentaethylene glycol monododecyl ether, Triton X-100, Cocamide monoethanolamine, Cocamide diethanolamine, Glycerol monostearate, Glycerol monolaurate, Sorbitan moonolaureate, Sorbitan monostearate, Tween 20, Tween 40, Tween 60, Tween 80, Alkyl polyglucosides, a poloxamer and optionally poloxamer 407.

74. The dry powder formulation of claim 73, wherein the surfactant is poloxamer.

75. The dry powder formulation of any one of claims 45-74, wherein the mRNA encodes a peptide.

76. The dry powder formulation of any one of claims 45-74, wherein the mRNA encodes a therapeutic protein.

77. The dry powder formulation of claim 76, wherein the therapeutic protein is CFTR.

78. The dry powder formulation of claim 76, wherein the therapeutic protein is OTC.

79. Use of the dry powder formulation of any one of claims 45-78 in the manufacture of a pharmaceutical composition for delivering mRNA *in vivo* to a subject in need thereof.

80. Use of the dry powder formulation of any one of claims 45-78 in the manufacture of a pharmaceutical composition for treating a disease or disorder in a patient, comprising that an effective dose of mRNA in the dry powder formulation is to be administered to the patient.

81. The use of claim 79 or 80, wherein the dry powder formulation is to be administered by inhalation.

82. The use of claim 79 or 80, wherein the dry powder formulation is to be administered by intranasal spray.

83. The use of claim 79 or 80, wherein the formulation is to be administered by a metered-dose inhaler.

84. The use of any one of claims 80-83, wherein the disease or disorder is selected from cystic fibrosis; asthma; COPD; emphysema; primary ciliary dyskinesia (CILD1) with or without situs inversus, or Kartagener syndrome; pulmonary fibrosis; Birt-Hogg-Dube syndrome; hereditary hemorrhagic telangiectasia; alpha-1 antitrypsin deficiency; Cytochrome b positive granulomatous diseases (CGD, X-lined); Cytochrome b positive granulomatous diseases, autosomal recessive; surfactant deficiency diseases, Pulmonary Surfactant Metabolism Dysfunction 1, Pulmonary Surfactant Metabolism Dysfunction 2, Pulmonary Surfactant Metabolism Dysfunction 3; Respiratory distress syndrome of prematurity; tuberculous tuberculosis, lung viral diseases, including influenza, Respiratory Syncytial Virus (RSV).

85. A method for manufacturing a dry powder formulation, the method comprising:

providing a mixture comprising an mRNA, one or more lipids and a polymer; and

spray-drying the mixture to form a plurality of particles.

86. The method of claim 85, wherein the one or more lipids are first mixed with the mRNA to form mRNA-loaded lipid nanoparticles before adding the polymer.

87. The method of claim 85, wherein the one or more lipids are mixed with the mRNA and the polymer in a single step to form mRNA-loaded lipid-polymer nanoparticles.

88. The method of any one of claims 85-87, further comprising adding to the mixture one or more excipients prior to spray drying.

89. The method of any one of claims 85-88, wherein the plurality of spray dried particles are characterized by one or more of the following:

a) less than 10% moisture content;
b) a fraction of fine particles with a volume median diameter less than 5 μm;
c) Z- average size range of 10- 3000 nm;
d) N/P ratio range from 1 to 20;
e) mRNA encapsulation efficiency of 80% or greater;
f) mRNA integrity of 90% or greater.

90. The method of any one of claims 85-89, wherein the mRNA encodes a protein or a peptide.

91. The method of any one of claims 85-90, wherein the mRNA encodes a peptide.

92. The method of any one of claims 85-90, wherein the mRNA encodes a therapeutic protein.

93. The method of claim 92, wherein the therapeutic protein is CFTR.

94. The method of claim 92, wherein the therapeutic protein is OTC.

95. A dry power formulation manufactured according to a method of any one of claims 85-94.

96. Use of the dry powder formulation of claim 95 in the manufacture of a pharmaceutical composition for treating a disease or disorder in a patient, comprising that an effective dose of mRNA in the dry powder formulation is to be administered to the patient.