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) comprising a plurality of spray-dried particles comprising mRNA encoding a protein or a peptide; one or more lipids, and one or more polymers comprising a polymethacrylate based polymer having a positively charged tertiary amine group with a methacrylic backbone, wherein the mRNA is encapsulated in one or more lipid nanoparticles (LNPs) comprising the one or more lipids; or wherein the mRNA is encapsulated in one or more lipid-polymer nanoparticles comprising the one or more lipids and the one or more polymers. In one example, 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 example, 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 example, 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 example, 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 yet another aspect, the present disclosure provides a 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 a 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, 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). In a further aspect, the present disclosure provides the dry powder formulation as described herein for use in therapy.

[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 a 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, 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).

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 a further aspect, the present disclosure provides a method for manufacturing a dry powder formulation, the method comprising: i. providing a mixture comprising mRNA, one or more lipids, and one or more polymers comprising a polymethacrylate based polymer having a positively charged tertiary amine group with a methacrylic backbone; ii. encapsulating the mRNA in one or more lipid nanoparticles (LNPs) comprising the one or more lipids; or encapsulating the mRNA in one or more lipid-polymer nanoparticles comprising the one or more lipids and the one or more polymers; and iii. spray-drying the mixture to form a plurality of spray-dried particles. In another example, 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.

We Claim:

1. A dry powder formulation for delivery of messenger RNA (mRNA) comprising a plurality of spray-dried particles comprising
    a. mRNA encoding a protein or a peptide;
    b. one or more lipids, and
    c. one or more polymers comprising a polymethacrylate based polymer having a positively charged tertiary amine group with a methacrylic backbone, wherein the mRNA is encapsulated in one or more lipid nanoparticles (LNPs) comprising the one or more lipids; or wherein the mRNA is encapsulated in one or more lipid-polymer nanoparticles comprising the one or more lipids and the one or more polymers.

2. The dry powder formulation of claim 1 comprising the mRNA encapsulated in the one or more LNPs.

3. The dry powder formulation of claim 1 comprising the mRNA encapsulated in the one or more lipid-polymer nanoparticles.

4. The dry powder formulation of claim 1, wherein the mRNA has an integrity of 90% or greater.

5. The dry powder formulation of claim 1, wherein the mRNA maintains an integrity of 90% or greater after storage at or under room temperature for 6 months or longer.

6. The dry powder formulation of claim 1, wherein the mRNA maintains an integrity of 90% or greater after storage at or under 4^{o}C for 6 months or longer.

7. The dry powder formulation of claim 1, wherein at least 20% of the plurality of spray-dried particles are fine particles.

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

9. The dry powder formulation of any one of claims 1-8, wherein the dry powder formulation is inhalable.

10. The dry powder formulation of any one of claims 1-8, wherein the formulation is nebulizable upon reconstitution.

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

12. The dry powder formulation of claim 11, wherein the one or more LNPs encapsulating the mRNA have a lipid: mRNA (N/P) ratio of 2 or 4.

13. The dry powder formulation of any one of claims 2-12, wherein the LNPs or lipid-polymer nanoparticles have an encapsulation efficiency of 80% or greater.

14. The dry powder formulation of any one of claims 2-13, wherein the one or more LNPs or lipid-polymer nanoparticles comprise one or more cationic lipids.

15. The dry powder formulation of claim 14, 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.

16. The dry powder formulation of any one of claims 2-15, wherein the one or more LNPs or lipid-polymer nanoparticles comprise one or more PEG-modified lipids.

17. The dry powder formulation of claim 2, wherein the LNPs are two-lipid component LNPs.

18. The dry powder formulation of any one of claims 2-16, wherein the one or more LNPs or lipid-polymer nanoparticles further comprise one or more neutral lipids or one or more cholesterol-based lipids.

19. The dry powder formulation of claim 2, wherein the LNPs are three-lipid component LNPs.

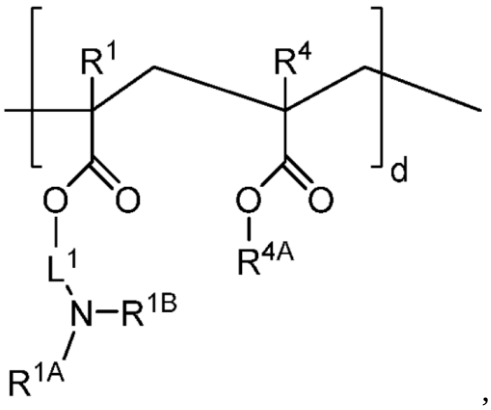
20. The dry powder formulation of any one of claims 1-19, wherein the one or more polymers constitute less than 20%, 15%, 12%, 10%, 9%, 8%, 7%, 6% or 5% of total weight.

21. The dry powder formulation of any one of claims 1-20, 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.

22. The dry powder formulation of any one of claims 1-21, wherein the one or more polymers constitute 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.

23. The dry powder formulation of any one of claims 1-22, 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.

24. The dry powder formulation of any one of claims 1-23, wherein the polymethacrylate based polymer having a positively charged tertiary amine group with a methacrylic backbone is represented by the following formula:



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wherein each of R^1 and R^4 is independently C_1-C_6 alkyl; each L^1 is independently C_2-C_6 alkylene; each of R^{1A} and R^{1B} is independently C_1-C_6 alkyl; each R^{4A} is independently C_1-C_6 alkyl; and d is an integer of 1-500.

25. The dry powder formulation of any one of claims 1-24, wherein the one or more polymers comprise Eudragit® EPO.

26. The dry powder formulation of any one of claims 1-25, 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.

27. The dry powder formulation of claim 26, wherein the sugar is mannitol.

28. The dry powder formulation of any one of claims 1-27, 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.

29. The dry powder formulation of claim 28, 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.

30. The dry powder formulation of claim 29, wherein the surfactant is poloxamer.

31. The dry powder formulation of any one of claims 1-30, wherein the mRNA encodes a peptide.

32. The dry powder formulation of any one of claims 1-30, wherein the mRNA encodes a therapeutic protein.

33. The dry powder formulation of claim 32, wherein the therapeutic protein is CFTR.

34. The dry powder formulation of claim 32, wherein the therapeutic protein is OTC.

35. Use of the dry powder formulation of any one of claims 1-34 in the manufacture of a pharmaceutical composition for delivering mRNA *in vivo* to a subject in need thereof.

36. Use of the dry powder formulation of any one of claims 1-34 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, 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).

37. The use of claim 35 or 36, wherein the dry powder formulation is to be administered by inhalation.

38. The use of claim 35 or 36, wherein the dry powder formulation is to be administered by intranasal spray.

39. The use of claim 35 or 36, wherein the dry powder formulation is to be administered by a metered-dose inhaler.

40. The dry powder formulation of any one of claims 1-34 for use in therapy.

41. A method for manufacturing a dry powder formulation, the method comprising:
    i. providing a mixture comprising mRNA, one or more lipids, and one or more polymers comprising a polymethacrylate based polymer having a positively charged tertiary amine group with a methacrylic backbone;
    ii. encapsulating the mRNA in one or more lipid nanoparticles (LNPs) comprising the one or more lipids; or encapsulating the mRNA in one or more lipid-polymer nanoparticles comprising the one or more lipids and the one or more polymers; and
    iii. spray-drying the mixture to form a plurality of spray-dried particles.

42. The method of claim 41, wherein the one or more lipids are first mixed with the mRNA to form mRNA-loaded lipid nanoparticles before adding the one or more polymers.

43. The method of claim 41, wherein the one or more lipids are mixed with the mRNA and the one or more polymers in a single step to form mRNA-loaded lipid-polymer nanoparticles.

44. The method of any one of claims 41-43, further comprising adding to the mixture one or more excipients prior to spray drying.

45. The method of any one of claims 41-44, 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.

46. The method of any one of claims 41-45, wherein the mRNA encodes a protein or a peptide.

47. The method of any one of claims 41-46, wherein the mRNA encodes a peptide.

48. The method of any one of claims 41-46, wherein the mRNA encodes a therapeutic protein.

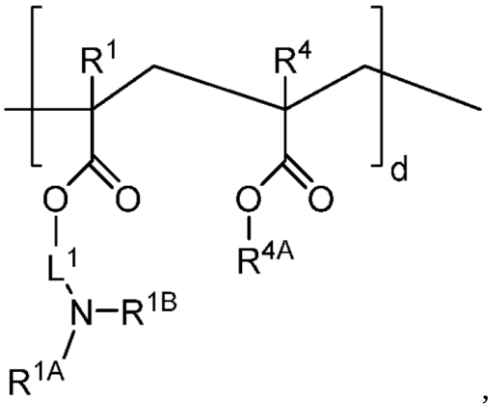
49. The method of claim 48, wherein the therapeutic protein is CFTR.

50. The method of claim 48, wherein the therapeutic protein is OTC.

51. A dry power formulation manufactured according to a method of any one of claims 41-50.

52. Use of the dry powder formulation of claim 51 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, 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).

53. The method of any one of claims 41-50, wherein the polymethacrylate based polymer having a positively charged tertiary amine group with a methacrylic backbone is represented by the following formula:



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wherein each of R^1 and R^4 is independently C_1-C_6 alkyl; each L^1 is independently C_2-C_6 alkylene; each of R^{1A} and R^{1B} is independently C_1-C_6 alkyl; each R^{4A} is independently C_1-C_6 alkyl; and d is an integer of 1-500.

54. The method of any one of claims 41-50, wherein the one or more polymers comprise Eudragit® EPO.