WHAT IS CLAIMED

1. A method of producing a lipid-encapsulated RNA nanoparticle, comprising the steps

a) flowing an aqueous solution comprising an RNA through a 1st tube having an inner diameter (ID) of from about 0.01 inches to about 0.08 inches; wherein a pH of the aqueous solution is in a range from about 3.0 to about 4.5 with an optional NaCl concentration of up to about 300 mM;

wherein the RNA comprises from about 6,000 to about 13,000 nucleotides;

b) flowing an ethanol solution comprising lipids through a 2nd tube having an ID of from about 0.01 inches to about 0.04 inches at a flow rate of about 0.2 to about 1 times a flow rate of the aqueous solution through the 1st tube, wherein the lipids comprise a cationic lipid; and

c) mixing the ethanol solution with the aqueous solution;

wherein the mixing produces an output solution flowing in the 1st tube comprising a turbulent flow of the RNA and the lipids in about 10% to 75% ethanol v/v; and

wherein the lipid-encapsulated RNA nanoparticles have a bilayer structure.

2. A method of producing a lipid-encapsulated RNA nanoparticle, comprising the steps

a) flowing an aqueous solution comprising an RNA through a 1st tube having a first inner diameter (ID);

wherein the RNA comprises from about 6,000 to about 13,000 nucleotides;

b) flowing an ethanol solution comprising lipids through a 2nd tube having a second inner diameter (ID), at a flow rate of about 0.2 to about 1 times a flow rate of the aqueous solution through the 1st tube, wherein the lipids comprise a cationic lipid; and

c) mixing the ethanol solution with the aqueous solution;

wherein the first ID and second ID and flow rates through the 1st tube and 2nd tube are selected to produce a shear force sufficiently low to preserve the integrity of the RNA;

wherein the mixing produces an output solution flowing in the 1st tube comprising a turbulent flow of the RNA and the lipids in between about 10% to 75% ethanol v/v; and

wherein the lipid-encapsulated RNA nanoparticles have a bilayer structure.

3. The method of claim 1 or 2, wherein the mixing comprises flowing the ethanol solution and the aqueous solution into a mixing module consisting of the 2nd tube perpendicularly joined to the 1st tube.

4. The method of claim 1 or 2, wherein the mixing comprises flowing the ethanol solution and the aqueous solution into a multi-inlet vortex mixer.

5. The method of any one of claims 1 to 4, wherein a concentration of RNA in the aqueous solution is in a range from about 85 micrograms/mL to about 2100 micrograms/mL.

6. The method of claim any one of claims 1 to 5, wherein a concentration of lipid in the ethanol solution is in a range from about 5.0 mg/mL to about 125 mg/mL.

7. The method of any one of claims 1 to 6, wherein the aqueous solution is pumped through the 1st tube by a 1st pump with a back pressure of not more than about 200 psi, and the ethanol solution is pumped through the 2nd tube by a 2nd pump.

8. The method of claim 1, wherein the 1st tube has an ID in a range from about 0.02 inches to about 0.03 inches and the 2nd tube has an ID in a range from about 0.01 inches to about 0.02 inches.

~~9. The method of claim 1, wherein the 1st tube has an ID of about 0.02 inches and the 2nd tube has an ID of about 0.01 inches.~~

~~10. The method of claim 1, wherein the 1st tube has an ID of about 0.03 inches and the 2nd tube has an ID of about 0.01 inches.~~

~~11. The method of claim 2, wherein the 1st tube has an ID in a range from about 0.01 inches to about 0.08 inches and the 2nd tube has an ID in a range from about 0.01 inches to about 0.04 inches.~~

~~12. The method of claim 2, wherein the 1st tube has an ID in a range from about 0.02 inches to about 0.03 inches and the 2nd tube has an ID in a range from about 0.01 inches to about 0.02 inches.~~

~~13.~~9. The method of any one of claims 1 to 8~~12~~, wherein the aqueous solution is pumped at a flow rate in a range from about 40 mL/min. to about 375 mL/min.

~~14. The method of any one of claims 1 to 13, wherein the ethanol solution is pumped at a flow rate in a range from about 10 mL/min. to about 75 mL/min.~~

~~15. The method of any one of claims 1 to 14, wherein the output solution has a total flow rate in a range from about 120 mL/min to about 300 mL/min.~~

~~16.~~10. The method of any one of claims 1 to ~~15~~9, wherein the aqueous, ethanol, and output solutions are maintained in a temperature range from about 10 °C to about 25 °C.

~~17.~~11. The method of any one of claims 1 to ~~16~~10, further comprising pumping a first dilution buffer and mixing the dilution buffer with the output solution by introducing the dilution buffer to the output solution to produce a first diluted output solution.

~~18.~~12. The method of claim ~~17~~11, further comprising pumping a second dilution buffer into the first diluted output solution thereby forming a final diluted output solution, wherein there is a delay between pumping the first dilution buffer and second dilution buffer.

~~19. The method of claim 18, wherein the delay is from about 0.1 to about 30 seconds and the delay is created by a length of tubing.~~

~~20.~~13. The method of ~~any one of claims 17 to 19~~claim 11 or claim 12, wherein the first dilution buffer comprises:

a) a buffering agent having a pH from about 5.5 to about 7.0; and

b) optionally a sodium chloride concentration up to about 100 mM~~.~~; and

~~21. The method of any one of claims 18 to 20, wherein~~ the second dilution buffer comprises

a) a buffering agent having a pH between about 7.4 and 8.0; and

b) optionally a sodium chloride concentration up to about 100 mM.

~~22.~~14. The method of ~~any one of claims 18 to 21~~claim 12 or 13, wherein the second buffer comprises sucrose up to about 15% w/v~~.~~

~~23. The method of any one of claims 18 to 22, wherein the second buffer comprises~~ an antioxidant up to about 0.5% w/v~~.~~

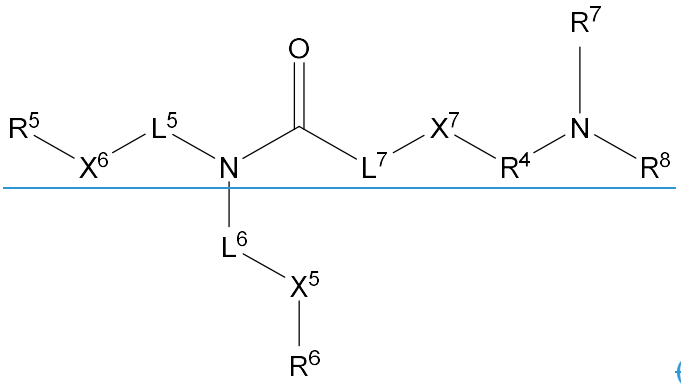
~~24. The method of any one of claims 18 to 23, wherein the second buffer comprises~~ up to 20 mM of a chelating agent, or any combination of the foregoing.

~~25.~~15. The method of any one of claims ~~17 to 24~~11 to 14, wherein the first diluted output solution comprises ~~about~~ 1.0% to ~~about~~ 10.0% ethanol.

~~26.~~16. The method of any one of claims ~~17 to 25~~11 to 15, wherein the first dilution buffer is pumped at a flow rate from about 80 mL/min. to about 900 mL/min~~.~~ and

~~27. The method of any one of claims 18 to 26, wherein~~ the second dilution buffer is pumped at a flow rate from about 240 mL/min to about 5400 mL/min.

~~28. The method of any one of claims 1 to 27, wherein the cationic lipid has a structure of Formula I:~~



~~(I)~~

~~or a pharmaceutically acceptable salt or solvate thereof, wherein~~

~~R^5 and R^6 are each independently selected from the group consisting of a linear or branched C_1-C_{31} alkyl, C_2-C_{31} alkenyl or C_2-C_{31} alkynyl and cholesteryl;~~

~~L^5 and L^6 are each independently selected from the group consisting of a linear C_1-C_{20} alkyl and C_2-C_{20} alkenyl;~~

~~X^5 is -C(O)O- or -OC(O)-;~~

~~X^6 is -C(O)O- or -OC(O)-;~~

~~X^7 is S or O;~~

~~L^7 is absent or lower alkyl;~~

~~R^4 is a linear or branched C_1-C_6 alkyl; and~~

~~R^7 and R^8 are each independently selected from the group consisting of a hydrogen and a linear or branched C_1-C_6 alkyl.~~

~~29.~~17. The method of any one of claims 1 to ~~28~~16, wherein the lipid-encapsulated RNA nanoparticle has an average particle size in a range from about 50 nm to about 120 nm.

~~30. The method of any one of claims 1 to 29, wherein the lipid-encapsulated RNA nanoparticle has an average particle size in a range from about 70 nm to about 90 nm.~~

~~31. The method of any one of claims 1 to 30, where the polydispersity lipid encapsulated RNA nanoparticles does not exceed about 0.2.~~

~~32.~~18. The method of any one of claims 1 to ~~31~~17, wherein the lipid portion of the lipid-encapsulated RNA nanoparticle further comprises one or more agents selected from the group consisting of a helper lipid, a cholesterol, and a PEG lipid conjugate.

~~33.~~19. The method of any one of claims 1 to ~~32~~18, wherein the RNA is self-replicating RNA.

~~34.~~20. The method of any one of claims 1 to ~~33~~19, further comprising lyophilizing the final diluted output solution.