What is Claimed

1. An optimized high-throughput screening method for manufacturing a lipid nanoparticle (LNP) preparation comprising:

a. obtaining a first solution comprising an aqueous phase;

b. obtaining a second solution comprising an organic phase and a plurality of molecules capable of self-assembly, and wherein said first and second solutions are intermixable;

c. dissolving at least one payload molecule into either the first or second solution;

d. using a robotic liquid handler to prepare and dispense said phases with varied compositions into a plurality of wells;

e. mixing said first and second solutions to obtain lipid nanoparticles encapsulating said payload using said robotic liquid handler under conditions suitable for LNP formation; wherein at least one of the following conditions are varied amongst different wells: type of self-assembly molecule, composition ratio of said self-assembly molecule; ratio and/or concentration of said self-assembly molecule to said payload, the selection of phase, buffer type and pH, the injection sequence, injection speed, mixing speed, volume, phase ratio, injection duration, and mixing duration;

f. measuring at least one of the following: encapsulation efficiency, particle size distribution, purification and particle recovery rate, and formulation stability of said LNPs;

g. determining the optimal parameters for manufacturing said LNP preparation; and

h. manufacturing said LNP preparation based on said optimal parameters.

2. The method of claim 1, wherein the payload is an oligonucleotide, an mRNA, a polypeptide, or a small molecule.

3. The method of claim 2, wherein the oligonucleotide is an antisense molecule, siRNA, or shRNA.

4. The method of claim 2 or 3, wherein the oligonucleotide is between 10 to 30 nucleotides in length.

5. The method of claim 2, wherein the size of mRNA is 500 to 3000 nucleotides in length.

6. The method of claim 2, wherein said polypeptide is between 1,000 Da and 10,000 Da, or wherein the small molecule is between 100 Da and 1000 Da.

7. The method of any one of claims 1-6, wherein the payload is dissolved in the first solution or wherein the payload is dissolved in the second solution.

8. The method of any one of claims 1-7, wherein the first solution is an aqueous buffer or comprises pH- and osmolality-controlled buffers.

9. The method of any one of claims 1-8, wherein the organic phase of the second solution comprises methanol or ethanol.

10. The method of any one of claims 1-9, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.

11. The method of claim 10, wherein the at least one species of lipid molecule is selected from the group consisting of a cationic lipid species, an ionizable lipid species, a non-cationic lipid species, a phospholipid species, and a non-phospholipid species.

12. The method of claim 10 or 11, wherein said second solution comprises more than one type of lipid.

13. The method of any one of claims 1-12, wherein the total concentration of lipid is varied, optionally the total concentration of lipid is varied between about 0.4 and about 4 mM.

14. The method of any one of claims 1-13, wherein the percentage of lipids that are PEGylated is varied, optionally the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.

15. The method of any one of claims 2-5, wherein when the payload is an oligonucleotide or an mRNA, the payload's N:P ratio is varied, optionally the N:P ratio is varied between about 0.5 to about 5.

16. The method of any of the preceding claims wherein the LNP is a polymer lipid nanoparticle, a liposome or a lipoprotein nanoparticle.

17. The method of any one of claims 1-16, wherein said first solution is injected into said second solution or said second solution is injected into said first solution.

18. A high-throughput method for optimizing the process for manufacturing a lipid nanoparticle (LNP) preparation comprising:

a. obtaining a first solution comprising an aqueous phase;

b. obtaining a second solution comprising an organic phase and a plurality of molecules capable of self-assembly, and wherein said first and second solutions are intermixable;

c. dissolving at least one payload molecule into either the first or second solution;

d. using a robotic liquid handler to prepare and dispense said phases with varied compositions into a plurality of wells;

e. mixing said first and second solutions to obtain lipid nanoparticles encapsulating said payload using said robotic liquid handler under conditions suitable for LNP formation; wherein at least one of the following conditions are varied amongst different wells: type of self-assembly molecule, composition ratio of said self-assembly molecule; ratio and/or concentration of said self-assembly molecule to said payload, the selection of phase, buffer type and pH, the injection sequence, injection speed, mixing speed, volume, phase ratio, injection duration, and mixing duration;

f. measuring at least one of the following: encapsulation efficiency, particle size distribution, purification and particle recovery rate, and formulation stability of said LNPs;

g. determining the optimal parameters for manufacturing said LNP preparation; and

h. manufacturing said LNP preparation based on said optimal parameters.

19. An optimized high-throughput method for encapsulating a payload in a liquid nanoparticle (LNP) preparation comprising:

a. obtaining a first solution comprising an aqueous phase;

b. obtaining a second solution comprising an organic phase and a plurality of molecules capable of self-assembly, and wherein said first and second solutions are intermixable;

c. dissolving at least one payload molecule into either the first or second solution;

d. using a robotic liquid handler to prepare and dispense said phases with varied compositions into a plurality of wells;

e. mixing said first and second solutions to obtain lipid nanoparticles encapsulating said payload using said robotic liquid handler under conditions suitable for LNP formation; wherein at least one of the following conditions are varied amongst different wells: type of self-assembly molecule, composition ratio of said self-assembly molecule; ratio and/or concentration of said self-assembly molecule to said payload, the selection of phase, buffer type and pH, the injection sequence, injection speed, mixing speed, volume, phase ratio, injection duration, and mixing duration;

f. measuring at least one of the following: encapsulation efficiency, particle size distribution, purification and particle recovery rate, and formulation stability of said LNPs;

g. determining the optimal parameters for manufacturing said LNP preparation; and

h. manufacturing said LNP preparation based on said optimal parameters.

20. An optimized lipid nanoparticle (LNP) manufactured by a process comprising the following steps:

a. obtaining a first solution comprising an aqueous phase;

b. obtaining a second solution comprising an organic phase and a plurality of molecules capable of self-assembly, and wherein said first and second solutions are intermixable;

c. dissolving at least one payload molecule into either the first or second solution;

d. using a robotic liquid handler to prepare and dispense said phases with varied compositions into a plurality of wells;

e. mixing said first and second solutions to obtain lipid nanoparticles encapsulating said payload using said robotic liquid handler under conditions suitable for LNP formation; wherein at least one of the following conditions are varied amongst different wells: type of self-assembly molecule, composition ratio of said self-assembly molecule; ratio and/or concentration of said self-assembly molecule to said payload, the selection of phase, buffer type and pH, the injection sequence, injection speed, mixing speed, volume, phase ratio, injection duration, and mixing duration;

f. measuring at least one of the following: encapsulation efficiency, particle size distribution, purification and particle recovery rate, and formulation stability of said LNPs;

g. determining the optimal parameters for manufacturing said LNP preparation; and

h. manufacturing said LNP preparation based on said optimal parameters.

21. The method of any one of claims 18-20, wherein the payload is an oligonucleotide, an mRNA, a polypeptide, or a small molecule.

22. The method of claim 21, wherein the oligonucleotide is an antisense molecule, siRNA, or shRNA.

23. The method of claim 21 or 22, wherein the oligonucleotide is between about 10 to about 30 nucleotides in length.

24. The method of claim 21, wherein the size of mRNA is 1 kb to 2 kb.

25. The method of claim 21, wherein said polypeptide is between 1,000 Da and 10,000 Da, or wherein the small molecule is between 100 Da and 1000 Da.

26. The method of any one of claims 18-25, wherein the payload is dissolved in the first solution or wherein the payload is dissolved in the second solution.

27. The method of any one of claims 18-26, wherein the first solution is an aqueous buffer or comprises pH- and osmolality-controlled buffers.

28. The method of any one of claims 18-27, wherein the organic phase of the second solution comprises methanol or ethanol.

29. The method of any one of claims 18-28, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.

30. The method of claim 29, wherein the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species.

31. The method of claim 29 or 30, wherein said second solution comprises more than one type of lipid.

32. The method of claim 29 or 30, wherein the total concentration of lipid is varied, optionally the total concentration of lipid is varied between about 0.4 and about 4 mM.

33. The method of claim 29 or 30, wherein the percentage of lipids that are PEGylated is varied, optionally the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.

34. The method of any one of claims 21-24, wherein when the payload is an oligonucleotide or an mRNA, the payload's N:P ratio is varied, optionally the N:P ratio is varied between about 0.5 to about 5.

35. The method of any one of claims 18-34, wherein the LNP is a polymer lipid nanoparticle, a liposome or a lipoprotein nanoparticle.

36. The method of any one of claims 18-35, wherein said first solution is injected into said second solution, or said second solution is injected into said first solution.

37. The method of any one of claims 1-36, wherein the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%.

38. The method of any one of claims 1-36, wherein the optimal parameters are those which produce a LNP with a mean diameter of 80-200 nm, having a unimodal size distribution, and a polydispersity of less than about 30%.

39. The method of any one of claims 1-36, wherein the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

40. An optimized high-throughput screening method for manufacturing a lipid nanoparticle (LNP) preparation comprising:

a. obtaining a first solution comprising an aqueous phase;

b. obtaining a second solution comprising an organic phase and a plurality of molecules capable of self-assembly, and wherein said first and second solutions are intermixable;

c. dissolving at least one payload molecule into either the first or second solution;

d. using a robotic liquid handler to prepare and dispense said phases with varied compositions into a plurality of wells;

e. mixing said first and second solutions to obtain lipid nanoparticles encapsulating said payload using said robotic liquid handler under conditions suitable for LNP formation; wherein at least one of the following conditions are varied amongst different wells: type of self-assembly molecule, composition ratio of said self-assembly molecule; ratio and/or concentration of said self-assembly molecule to said payload, the selection of phase, buffer type and pH, the injection sequence, injection speed, mixing speed, volume, phase ratio, injection duration, and mixing duration;

f. measuring at least one of the following: encapsulation efficiency, particle size distribution, purification and particle recovery rate, and formulation stability of said LNPs;

g. determining the optimal parameters for manufacturing said LNP preparation; and

h. manufacturing said LNP preparation based on said optimal parameters;

and wherein the encapsulation efficiency is optimized by measuring the charge ratio of ionizable lipid / oligonucleotide.

41. The method of claim 40, wherein the oligonucleotide is an antisense molecule, a siRNA or a shRNA.

42. The method of claim 41, wherein the oligonucleotide is between 10 to 30 nucleotides in length.

43. The method of claim 40, wherein the payload is an mRNA, a polypeptide, or a small molecule.

44. The method of claim 43, wherein the size of mRNA is 500 to 3000 nucleotides in length, wherein said polypeptide is between 1,000 Da and 10,000 Da, or wherein the small molecule is between 100 Da and 1000 Da.

45. The method of any one of claims 40-44, wherein the payload is dissolved in the first solution or wherein the payload is dissolved in the second solution.

46. The method of any one of claims 40-45, wherein the first solution is an aqueous buffer or comprises pH- and osmolality-controlled buffers.

47. The method of any one of claims 40-46, wherein the organic phase of the second solution comprises methanol or ethanol.

48. The method of any one of claims 40-47, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.

49. The method of claim 48, wherein the at least one species of lipid molecule is selected from the group consisting of a cationic lipid species, an ionizable lipid species, a non-cationic lipid species, a phospholipid species, and a non-phospholipid species.

50. The method of claim 49, wherein said second solution comprises more than one type of lipid.

51. The method of any one of claims 40-50, wherein the total concentration of lipid is varied, optionally, the total concentration of lipid is varied between about 0.4 and about 4 mM.