CLAIMS

What is Claimed is:

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.

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

4. The method of claim 1, wherein the payload is a polypeptide or a small molecule.

5. The method of claim 1, wherein the payload is dissolved in the first solution.

6. The method of claim 1, wherein the payload is dissolved in the second solution.

7. The method of claim 1, wherein the first solution is an aqueous buffer.

8. The method of claim 1, wherein the first solution comprises pH- and osmolality-controlled buffers.

9. The method of claim 1, wherein the organic phase of the second solution comprises methanol or ethanol.

10. The method of claim 1, 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 claim 1, wherein the total concentration of lipid is varied.

14. The method of claim 13, wherein the total concentration of lipid is varied between about 0.4 and about 4 mM.

15. The method of claim 1, wherein the percentage of lipids that are PEGylated is varied.

16. The method of claim 15, wherein the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.

17. The method of claim 2 or 3, wherein the payload's N:P ratio is varied.

18. The method of claim 17, wherein the N:P ratio is varied between about 0.5 to about 5.

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

20. The method of any of the preceding claims, wherein the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%.

21. The method of any of claims 1-19, wherein the optimal parameters are those which produce a LNP with a mean diameter of 80-200 nm, having an unimodal size distribution, and a polydispersity of less than about 30%.

22. A LNP preparation for administration to a patient in need thereof for use in therapy, wherein said LNP preparation is manufactured by the method of any one of claims 1-21.

23. An optimized lipid nanoparticle (LNP) preparation manufactured by the process of any one of claims 1-21.

24. The LNP preparation of claim 22 or 23, wherein the LNP preparation comprises polymer lipid nanoparticles, liposomes, or lipoprotein nanoparticles.

25. The optimized LNP preparation of claim 23, wherein the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

26. A workflow for HTS screening of a plurality of parameters for LNP formation, comprising:

(i) a robotic liquid handler;

(ii) at least one instrument capable of measuring desired LNP characteristics; and

(iii) at least one microplate comprising a plurality of microwells;

wherein said robotic liquid handler is capable of injecting a plurality of solutions into each of said microwells;

wherein said parameters are systematically varied between microwells; and

wherein said desired LNP characteristics are capable of being measured for each microwell.