with cationic lipids. Schroeder *et al.*, 2010, J Intern Med 267(1): 9-21; Cullis *et al.*, 2017, Mol Ther 25(7):1467-1475. The downstream performance of LNPs is also highly governed by their quality attributes. Screening of various levels of these parameters therefore highly demands a high-throughput approach with facile procedures and multiple analytical outputs.

[0004] LNP structure and cargo delivery are regulated by four major components: ionizable lipids, helper phospholipids, cholesterol, and polyethylene glycol-lipids (PEG-lipids). Cationic ionizable lipids promote the encapsulation of negatively charged nucleic acids during LNP formulation and aid in cytosolic delivery of cargo at endosomal pH range 5.5-6.5. Helper lipids and cholesterol increase structural stability, promote membrane fusion, and enhance endosomal escape of the LNPs. The effect of PEG-lipid addition is multi-faceted and contributes to what has been termed the 'PEG-dilemma'. PEG-lipids are necessary to control particle size during self-assembly and prevent particle aggregation. However, the hydrophilic PEG corona can hinder interactions between the particle surface and the lipophilic cell membrane, resulting in poor cellular internalization. The presence of PEG can also prevent surface binding of transport proteins necessary for LNP cellular internalization via receptor-mediated endocytosis. Furthermore, PEG-lipids also prolong the *in vivo* LNP circulation time by acting as a steric barrier to the adsorption of plasma proteins, including opsonins. While extending half-life increases therapeutic exposure, increasing LNP circulation can induce anti-PEG antibody generation leading to adverse allergic reactions.

[0005] Thus, achieving effective intracellular delivery is highly dependent on understanding the effect of multivariate PEG-lipids to overcome the PEG dilemma. Previous studies indicate tuning PEG size, architecture, content, carbon tail type, and length modulates LNP efficacy.

SUMMARY

[0006] To address the needs of screening and optimization of lipid-based nanomedicines, the present disclosure provides for a high-throughput screening (HTS) workflow for the preparation of such lipid-based nanoparticles encapsulating various therapeutic payloads. In various examples, the present discosure provides an optimized solvent-injection method for facile self-assembly of LNPs using a robotic liquid handler. In various examples, optimal lipid composition, total lipid concentration, and loading amount of a payload are described.

[0007] In one aspect, the present disclosure relates to 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.

[0008] As noted herein, Lipid Nanoparticles (LNPs) are increasingly utilized to improve the delivery efficiency and therapeutic efficacy of nucleic acids. It has been found that various formulation parameters can affect the quality attributes of these nanoparticle formulations. There is, however, currently a lack of effective systemic screening approaches to address these challenges. In accordance with the discosure, an automated high-throughput screening (HTS) workflow has been developed for streamlined preparation and analytical characterization of LNPs loaded with, e.g., antisense oligonucleotides (ASOs). In accordance with the discosure, this characterization can be performed in a full 96-well plate within as little as 3 hours.

[0009] In accordance with the discosure, ASO-loaded LNPs were formulated by an automated solvent-injection method using a robotic liquid handler, and assessed for particle size distribution, encapsulation efficiency, and stability with different formulation compositions and ASO loadings. The results described herein indicate that the PEGylated lipid content significantly affected the particle size distribution, while the ionizable lipid / ASO charge ratio impacted the encapsulation efficiency of the ASOs. Furthermore, results from our HTS approach correlated with those from the state-of-the-art scale-up method using a microfluidic formulator, thus providing a novel method for robust formulation development and design of experiment methods. This method can reduce material usage by ~10-fold, thereby improving analytical outputs and accumulation of information by ~100 fold.

[0010] A novel HTS workflow has been developed to prepare ASO-LNPs and analyze the relationship between their PEG-lipid content and particle size distribution. Using a high-throughput liquid handler, libraries of varying ASO-LNPs containing multiple PEG-lipids across the phosphoglyceride, diglyceride, and ceramide families were made and formulated at varying PEG-lipid molar ratios (1-5mol%). The impact of PEG-lipid parameters including molecular weight (mw), carbon-tail length, and molar ratios is described herein. Additionally, a number of additional PEG-lipid variables are described herein, including PEG architecture, lipid tail saturation, PEG-lipid charge, and linker chemistry.

[0011] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 500-3000 nucleotides. In various examples, the payload is a polypeptide. In various examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0012] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0013] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic or ionizable lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0014] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0015] In one aspect, the present disclosure relates to 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.

[0016] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 1 kb to about 2 kb. In various examples, the payload is a polypeptide. In various

examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0017] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0018] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0019] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0020] In one aspect, the present disclosure relates to 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.

[0021] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 1 kb to about 2 kb. In various examples, the payload is a polypeptide. In various examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0022] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0023] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In

various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0024] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0025] In various examples, the present disclosure relates to a method of administering a LNP preparation to a patient in need thereof, wherein said LNP preparation is manufactured by: 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.

[0026] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 1 kb to about 2 kb. In various examples, the payload is a polypeptide. In various examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0027] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0028] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0029] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size

distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0030] In various examples, the present disclosure relates to 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.

[0031] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 1 kb to about 2 kb. In various examples, the payload is a polypeptide. In various examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0032] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution

comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0033] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0034] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0035] In various examples, the present disclosure relates to 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.

[0036] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 1 kb to about 2 kb. In various examples, the payload is a polypeptide. In various examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0037] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0038] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0039] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein

nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0040] In various examples, the present disclosure relates to 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.

[0041] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 1 kb to about 2 kb. In various examples, the payload is a polypeptide. In various examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various

examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0042] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0043] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0044] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0045] In one aspect, the present disclosure relates to 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.

[0046] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 1 kb to about 2 kb. In various examples, the payload is a polypeptide. In various examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0047] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0048] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various

examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0049] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0050] In various examples, the present disclosure relates to 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.

[0051] In various examples, the payload is an oligonucleotide. In various examples, the oligonucleotide is an antisense molecule. In various examples, the oligonucleotide is a siRNA. In various examples, the oligonucleotide is a shRNA. In various

examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various examples, the payload is an mRNA. In various examples, the size of mRNA is about 1 kb to about 2 kb. In various examples, the payload is a polypeptide. In various examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various examples, the payload is a small molecule. In various examples, the small molecule is between about 100 Da and 1000 Da.

[0052] In various examples, the payload is dissolved in the first solution. In various examples, the payload is dissolved in the second solution. In various examples, the first solution is an aqueous buffer. In various examples, the first solution comprises pH- and osmolality-controlled buffers. In various examples, the organic phase of the second solution comprises methanol. In various examples, the organic phase of the second solution comprises ethanol.

[0053] In various examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various examples, the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species. In various examples, said second solution comprises more than one type of lipid. In various examples, the total concentration of lipid is varied. In various examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various examples, the percentage of lipids that are PEGylated is varied. In various examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various examples, the payload's N:P ratio is varied. In various examples, the N:P ratio is varied between about 0.5 to about 5.

[0054] In various examples, the LNP is a polymer lipid nanoparticle. In various examples, the LNP is a liposome. In various examples, the LNP is a lipoprotein nanoparticle. In various examples, said first solution is injected into said second solution. In various examples, said second solution is injected into said first solution. In various examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various examples, 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%. In various examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0054a] In one aspect, the present disclosure relates to 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.

[0055] In various examples, the present disclosure relates to 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.

[0056] In various examples, the plurality of parameters are selected from total lipid content, type of self-assembly molecule; the composition ratio of said self-assembly molecule; the ratio and/or concentration of said self-assembly molecule to said payload; the selection of phase, the buffer type and pH, the injection sequence, volume, and speed, and the mixing duration. In various examples, said desired LNP characteristics are selected from the group consisting of: average particle size, particle size distribution, encapsulation efficiency, and particle stability. In various examples said instrument is capable of either dynamic light scattering (DLS), ultraviolet-visible (UV-Vis), or fluorescence spectroscopy.

BRIEF DESCRIPTION OF THE FIGURES

[0057] **FIGS. 1A-1F** show data from the high-speed, ethanol-to-buffer injection followed by multiple rounds of mixing produced uniform LNPs with high ASO loading. LNPs composed of 0.4 μmol of total lipids and 1.5 mol% of DSPE-PEG2000 were mixed with ASO-1 under the N/P ratio of 1 using different mixing conditions. A TECAN® robot was used to investigate reverse injection sequences (ethanol-to-buffer or buffer-to-ethanol) at a speed of 0.1, 0.5, or 0.9 ml/s followed by 10 mixing repeats (FIGs. 1A-1C), or the ethanol-to-buffer injection at a speed of 0.5 or 0.9 ml/s followed by 10 or 20 mixing repeats (FIGs. 1D-1F). Particle size (FIGs. 1A and 1D) and polydispersity (FIGs. 1B and 1E) were measured by dynamic light scattering (DLS). Free ASO-1 was measured by OD260 and calculated for encapsulation efficiency (FIGs. 1C and 1F). Results are mean ± SD, n = 3; ns, not significant, **** $P < 0.0001$, analyzed by (FIGs. 1A-1C) two-way or one-way (FIGs. 1D-1F) ANOVA followed by Tukey's multiple comparisons.

[0058] **FIG. 2** shows the HTS workflow for ASO-loaded LNP formulations. Ninety six samples (32 conditions, n = 3) varying with 4 levels of lipid composition, 2 levels of total lipid concentration, and 4 levels of ASO loading amount were prepared by the automated solvent-injection method using a TECAN® liquid handler, followed by characterization of particle size distributions by DLS and ASO encapsulation by absorbance at 260 nm. A representative LEA