quality attributes. Screening of various levels of these parameters therefore highly demands a high-throughput approach with facile procedures and multiple analytical outputs.

SUMMARY ~~OF THE INVENTION~~

[0003] 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 ~~embodiment~~examples, the present ~~invention~~disclosure provides an optimized solvent-injection method for facile self-assembly of LNPs using a robotic liquid handler. In various ~~embodiment~~examples, optimal lipid composition, total lipid concentration, and loading amount of a payload are described.

[0004] In ~~various embodiments~~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.

[0005] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various ~~embodiment~~examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various ~~embodiment~~examples, the payload is an mRNA. In various ~~embodiment~~examples, the size of mRNA is about 500-3000 nucleotides. In various ~~embodiment~~examples, the payload is a polypeptide. In various ~~embodiment~~examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various

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

[0006] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0007] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises more than one type of lipid. In various ~~embodiment~~examples, the total concentration of lipid is varied. In various ~~embodiment~~examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated is varied. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various ~~embodiment~~examples, the payload's N:P ratio is varied. In various ~~embodiment~~examples, the N:P ratio is varied between about 0.5 to about 5.

[0008] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0009] In various ~~embodiment~~examples, 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.

[0010] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various ~~embodiment~~examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various ~~embodiment~~examples, the payload is an mRNA. In various ~~embodiment~~examples, the size of mRNA is about 1 kb to about 2 kb. In various ~~embodiment~~examples, the payload is a polypeptide. In various ~~embodiment~~examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various ~~embodiment~~examples, the payload is a small molecule. In various ~~embodiment~~examples, the small molecule is between about 100 Da and 1000 Da.

[0011] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0012] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises more than one type of lipid. In various ~~embodiment~~examples, the total concentration of lipid is varied. In various ~~embodiment~~examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated is varied. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated are varied between about 0.5% to

about 5% of the total lipid composition. In various ~~embodiment~~examples, the payload's N:P ratio is varied. In various ~~embodiment~~examples, the N:P ratio is varied between about 0.5 to about 5.

[0013] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0014] In various ~~embodiment~~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.

[0015] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various ~~embodiment~~examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various ~~embodiment~~examples, the payload is an mRNA. In various ~~embodiment~~examples, the size of mRNA is

about 1 kb to about 2 kb. In various ~~embodiment~~examples, the payload is a polypeptide. In various ~~embodiment~~examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various ~~embodiment~~examples, the payload is a small molecule. In various ~~embodiment~~examples, the small molecule is between about 100 Da and 1000 Da.

[0016] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0017] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises more than one type of lipid. In various ~~embodiment~~examples, the total concentration of lipid is varied. In various ~~embodiment~~examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated is varied. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various ~~embodiment~~examples, the payload's N:P ratio is varied. In various ~~embodiment~~examples, the N:P ratio is varied between about 0.5 to about 5.

[0018] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0019] In one aspect, the present disclosure relates to a LNP preparation for administration to a patient in need thereof for use in therapy, wherein said LNP preparation is manufactured by the method as disclosed herein. In various ~~embodiment~~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.

[0020] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various ~~embodiment~~examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various ~~embodiment~~examples, the payload is an mRNA. In various ~~embodiment~~examples, the size of mRNA is about 1 kb to about 2 kb. In various ~~embodiment~~examples, the payload is a polypeptide. In various ~~embodiment~~examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various ~~embodiment~~examples, the payload is a small molecule. In various ~~embodiment~~examples, the small molecule is between about 100 Da and 1000 Da.

[0021] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0022] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises more than one type of lipid. In various ~~embodiment~~examples, the total concentration of lipid is varied. In various ~~embodiment~~examples, the total concentration of lipid is varied between about 0.4 and about 4

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

[0023] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0024] In various ~~embodiment~~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.

[0025] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various

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

[0026] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0027] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises more than one type of lipid. In various ~~embodiment~~examples, the total concentration of lipid is varied. In various ~~embodiment~~examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated is varied. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various ~~embodiment~~examples, the payload's N:P ratio is varied. In various ~~embodiment~~examples, the N:P ratio is varied between about 0.5 to about 5.

[0028] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0029] In various ~~embodiment~~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.

[0030] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various ~~embodiment~~examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various ~~embodiment~~examples, the payload is an mRNA. In various ~~embodiment~~examples, the size of mRNA is about 1 kb to about 2 kb. In various ~~embodiment~~examples, the payload is a polypeptide. In various ~~embodiment~~examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various ~~embodiment~~examples, the payload is a small molecule. In various ~~embodiment~~examples, the small molecule is between about 100 Da and 1000 Da.

[0031] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0032] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises

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

[0033] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0034] In various ~~embodiment~~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.

[0035] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various ~~embodiment~~examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various ~~embodiment~~examples, the payload is an mRNA. In various ~~embodiment~~examples, the size of mRNA is about 1 kb to about 2 kb. In various ~~embodiment~~examples, the payload is a polypeptide. In various ~~embodiment~~examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various ~~embodiment~~examples, the payload is a small molecule. In various ~~embodiment~~examples, the small molecule is between about 100 Da and 1000 Da.

[0036] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0037] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises more than one type of lipid. In various ~~embodiment~~examples, the total concentration of lipid is varied. In various ~~embodiment~~examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated is varied. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various ~~embodiment~~examples, the payload's N:P ratio is varied. In various ~~embodiment~~examples, the N:P ratio is varied between about 0.5 to about 5.

[0038] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size

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

[0039] In one aspect, the present disclosure relates to an optimized lipid nanoparticle (LNP) preparation manufactured by the process as disclosed herein. In various ~~embodiment~~examples, 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.

[0040] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various ~~embodiment~~examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various ~~embodiment~~examples, the payload is an mRNA. In various ~~embodiment~~examples, the size of mRNA is about 1 kb to about 2 kb. In various ~~embodiment~~examples, the payload is a polypeptide. In various ~~embodiment~~examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various ~~embodiment~~examples, the payload is a small molecule. In various ~~embodiment~~examples, the small molecule is between about 100 Da and 1000 Da.

[0041] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0042] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises more than one type of lipid. In various ~~embodiment~~examples, the total concentration of lipid is varied. In various ~~embodiment~~examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated is varied. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various ~~embodiment~~examples, the payload's N:P ratio is varied. In various ~~embodiment~~examples, the N:P ratio is varied between about 0.5 to about 5.

[0043] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0044] In various ~~embodiment~~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.

[0045] In various ~~embodiment~~examples, the payload is an oligonucleotide. In various ~~embodiment~~examples, the oligonucleotide is an antisense molecule. In various ~~embodiment~~examples, the oligonucleotide is a siRNA. In various ~~embodiment~~examples, the oligonucleotide is a shRNA. In various ~~embodiment~~examples, the oligonucleotide is between about 10 to about 30 nucleotides in length. In various ~~embodiment~~examples, the payload is an mRNA. In various ~~embodiment~~examples, the size of mRNA is about 1 kb to about 2 kb. In various ~~embodiment~~examples, the payload is a polypeptide. In various ~~embodiment~~examples, said polypeptide is between about 1,000 Da and about 10,000 Da. In various ~~embodiment~~examples, the payload is a small molecule. In various ~~embodiment~~examples, the small molecule is between about 100 Da and 1000 Da.

[0046] In various ~~embodiment~~examples, the payload is dissolved in the first solution. In various ~~embodiment~~examples, the payload is dissolved in the second solution. In various ~~embodiment~~examples, the first solution is an aqueous buffer. In various ~~embodiment~~examples, the first solution comprises pH- and osmolality-controlled buffers. In various ~~embodiment~~examples, the organic phase of the second solution comprises methanol. In various ~~embodiment~~examples, the organic phase of the second solution comprises ethanol.

[0047] In various ~~embodiment~~examples, the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule. In various ~~embodiment~~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 ~~embodiment~~examples, said second solution comprises more than one type of lipid. In various ~~embodiment~~examples, the total concentration of lipid is varied. In various ~~embodiment~~examples, the total concentration of lipid is varied between about 0.4 and about 4 mM. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated is varied. In various ~~embodiment~~examples, the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition. In various ~~embodiment~~examples, the payload's N:P ratio is varied. In various ~~embodiment~~examples, the N:P ratio is varied between about 0.5 to about 5.

[0048] In various ~~embodiment~~examples, the LNP is a polymer lipid nanoparticle. In various ~~embodiment~~examples, the LNP is a liposome. In various ~~embodiment~~examples, the LNP is a lipoprotein nanoparticle. In various ~~embodiment~~examples, said first solution is injected into said second solution. In various ~~embodiment~~examples, said second solution is injected into said first solution. In various ~~embodiment~~examples, the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%. In various ~~embodiment~~examples, 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%. In various ~~embodiment~~examples, the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

[0049] In ~~various embodiments~~one aspect, 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.

[0050] In various ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~examples said instrument is capable of either dynamic light scattering (DLS), ultraviolet-visible (UV-Vis), or fluorescence spectroscopy.

BRIEF DESCRIPTION OF THE FIGURES

[0051] **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.

[0052] **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