CLAIMS

What is Claimed is:

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

a. obtaining a first solution comprising an aqueous phase;

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

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

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

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

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

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

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

2. The method of claim 1, wherein the payload is an oligonucleotide.

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

~~4. The method of claim 2, wherein the oligonucleotide is a siRNA.~~

~~5. The method of claim 3, wherein the oligonucleotide is a shRNA.~~

~~6. The method of claims 2 through 5, wherein the oligonucleotide is between about 10 to about 30 nucleotides in length.~~

~~7. The method of claim 1, wherein the payload is an mRNA.~~

~~8. The method of claim 7, wherein the size of mRNA is about 500 to about 3000 nucleotides in length.~~

~~9~~4. The method of claim 1, wherein the payload is a polypeptide or a small molecule.

~~10. The method of claim 9, wherein said polypeptide is between about 1,000 Da and about 10,000 Da.~~

~~11. The method of claim 1, wherein the payload is a small molecule.~~

~~12. The method of claim 11, wherein the small molecule is between about 100 Da and 1000 Da.~~

~~13~~5. The method of claim 1, wherein the payload is dissolved in the first solution.

~~14~~6. The method of claim 1, wherein the payload is dissolved in the second solution.

~~15~~7. The method of claim 1, wherein the first solution is an aqueous buffer.

~~16~~8. The method of claim 1, wherein the first solution comprises pH- and osmolality-controlled buffers.

~~17~~9. The method of claim 1, wherein the organic phase of the second solution comprises methanol or ethanol.

~~18. The method of claim 1, wherein the organic phase of the second solution comprises ethanol.~~

~~19~~10. The method of claim 1, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.

~~20~~11. The method of claim ~~19~~10, wherein the at least one species of lipid molecule is selected from the group consisting of a cationic lipid species, an ionizable lipid species, a non-cationic lipid species, a phospholipid species, and a non-phospholipid species.

~~21~~12. The method of claim ~~19~~ 10 or ~~20~~11, wherein said second solution comprises more than one type of lipid.

~~22~~13. The method of claim 1, wherein the total concentration of lipid is varied.

~~23~~14. The method of claim ~~22~~13, wherein the total concentration of lipid is varied between about 0.4 and about 4 mM.

~~24~~15. The method of claim 1, wherein the percentage of lipids that are PEGylated is varied.

~~25~~16. The method of claim ~~24~~15, wherein the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.

~~26~~17. The method of ~~any one of~~ claim~~s~~ 2~~-8~~ or 3, wherein the payload's N:P ratio is varied.

~~27~~18. The method of claim ~~26~~17, wherein the N:P ratio is varied between about 0.5 to about 5.

~~28~~19. The method of any of the preceding claims, wherein the LNP is a polymer lipid nanoparticle, a liposome, or a lipoprotein nanoparticle.

~~29. The method of claims 1-27, wherein the LNP is a liposome.~~

~~30. The method of claims 1-27, wherein the LNP is a lipoprotein nanoparticle.~~

~~31. The method of claim 1, wherein said first solution is injected into said second solution.~~

~~32. The method of claim 1, wherein said second solution is injected into said first solution.~~

~~33~~20. The method of any of the preceding claims, wherein the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%.

~~34~~21. The method of any of claims 1-~~32~~19, wherein the optimal parameters are those which produce a LNP with a mean diameter of 80-200 nm, having an unimodal size distribution, and a polydispersity of less than about 30%.

~~35. The method of any one of claims 1-32, wherein the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.~~

~~36. 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.~~

~~37. The method of claim 36, wherein the payload is an oligonucleotide.~~

~~38. The method of claim 37, wherein the oligonucleotide is an antisense molecule.~~

~~39. The method of claim 37, wherein the oligonucleotide is a siRNA.~~

~~40. The method of claim 38, wherein the oligonucleotide is a shRNA.~~

~~41. The method of claims 37 through 42, wherein the oligonucleotide is between about 10 to about 30 nucleotides in length.~~

~~42. The method of claim 36, wherein the payload is an mRNA.~~

~~43. The method of claim 42, wherein the size of mRNA is about 1 kb to about 2 kb.~~

~~44. The method of claim 36, wherein the payload is a polypeptide.~~

~~45. The method of claim 45, wherein said polypeptide is between about 1,000 Da and about 10,000 Da.~~

~~46. The method of claim 36, wherein the payload is a small molecule.~~

~~47. The method of claim 46, wherein the small molecule is between about 100 Da and 1000 Da.~~

~~48. The method of claim 36, wherein the payload is dissolved in the first solution.~~

~~49. The method of claim 36, wherein the payload is dissolved in the second solution.~~

~~50. The method of claim 36, wherein the first solution is an aqueous buffer.~~

~~51. The method of claim 36, wherein the first solution comprises pH- and osmolality-controlled buffers.~~

~~52. The method of claim 36, wherein the organic phase of the second solution comprises methanol.~~

~~53. The method of claim 36, wherein the organic phase of the second solution comprises ethanol.~~

~~54. The method of claim 36, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.~~

~~55. The method of claim 54, wherein the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species.~~

~~56. The method of claim 54 or 55, wherein said second solution comprises more than one type of lipid.~~

~~57. The method of claim 36, wherein the total concentration of lipid is varied.~~

~~58. The method of claim 57, wherein the total concentration of lipid is varied between about 0.4 and about 4 mM.~~

~~59. The method of claim 54 or 55, wherein the percentage of lipids that are PEGylated is varied.~~

~~60. The method of claim 59, wherein the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.~~

~~61. The method of any one of claims 37-42, wherein the payload's N:P ratio is varied.~~

~~62. The method of claim 61, wherein the N:P ratio is varied between about 0.5 to about 5.~~

~~63. The method of any one of claims 36-62, wherein the LNP is a polymer lipid nanoparticle.~~

~~64. The method of claims 36-62, wherein the LNP is a liposome.~~

~~65. The method of claims 36-62, wherein the LNP is a lipoprotein nanoparticle.~~

~~66. The method of claim 36, wherein said first solution is injected into said second solution.~~

~~67. The method of claim 36, wherein said second solution is injected into said first solution.~~

68. The method of any one of claims 36-67, wherein the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%.

69. The method of any one of claims 36-67, wherein the optimal parameters are those which produce a LNP with a mean diameter of 80-200 nm, having an unimodal size distribution, and a polydispersity of less than about 30%.

70. The method of any one of claims 36-67, wherein the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

71. 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.

72. The method of claim 71, wherein the payload is an oligonucleotide.

73. The method of claim 72, wherein the oligonucleotide is an antisense molecule.

~~74. The method of claim 73, wherein the oligonucleotide is a siRNA.~~

~~75. The method of claim 73, wherein the oligonucleotide is a shRNA.~~

~~76. The method of claims 72 through 75, wherein the oligonucleotide is between about 10 to about 30 nucleotides in length.~~

~~77. The method of claim 71, wherein the payload is an mRNA.~~

~~78. The method of claim 77, wherein the size of mRNA is about 1 kb to about 2 kb.~~

~~79. The method of claim 71, wherein the payload is a polypeptide.~~

~~80. The method of claim 79, wherein said polypeptide is between about 1,000 Da and about 10,000 Da.~~

~~81. The method of claim 71, wherein the payload is a small molecule.~~

~~82. The method of claim 81, wherein the small molecule is between about 100 Da and 1000 Da.~~

~~83. The method of claim 71, wherein the payload is dissolved in the first solution.~~

~~84. The method of claim 71, wherein the payload is dissolved in the second solution.~~

~~85. The method of claim 71, wherein the first solution is an aqueous buffer.~~

~~86. The method of claim 71, wherein the first solution comprises pH- and osmolality-controlled buffers.~~

~~87. The method of claim 71, wherein the organic phase of the second solution comprises methanol.~~

~~88. The method of claim 71, wherein the organic phase of the second solution comprises ethanol.~~

~~89. The method of claim 71, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.~~

~~90. The method of claim 89, wherein the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species.~~

~~91. The method of claim 89 or 90, wherein said second solution comprises more than one type of lipid.~~

~~92. The method of claim 89 or 90, wherein the total concentration of lipid is varied.~~

~~93. The method of claim 92, wherein the total concentration of lipid is varied between about 0.4 and about 4 mM.~~

~~94. The method of claim 89 or 90, wherein the percentage of lipids that are PEGylated is varied.~~

~~95. The method of claim 94, wherein the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.~~

~~96. The method of any one of claims 72-78, wherein the payload's N:P ratio is varied.~~

~~97. The method of claim 96, wherein the N:P ratio is varied between about 0.5 to about 5.~~

~~98. The method of any one of claims 71-97, wherein the LNP is a polymer lipid nanoparticle.~~

~~99. The method of claims 71-97, wherein the LNP is a liposome.~~

~~100. The method of claims 71-97, wherein the LNP is a lipoprotein nanoparticle.~~

~~101. The method of claim 71, wherein said first solution is injected into said second solution.~~

~~102. The method of claim 71, wherein said second solution is injected into said first solution.~~

~~103. The method of any one of claims 71-102, wherein the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%.~~

~~104. The method of any one of claims 71-102, wherein the optimal parameters are those which produce a LNP with a mean diameter of 80-200 nm, having an unimodal size distribution, and a polydispersity of less than about 30%.~~

~~105. The method of any one of claims 71-102, wherein the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.~~

~~10622~~. A ~~method of administering a~~ LNP preparation for administration to a patient in need thereof for use in therapy, wherein said LNP preparation is manufactured by the method of any one of claims 1-21.~~:~~

~~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.~~

~~107. The method of claim 106, wherein the payload is an oligonucleotide.~~

~~108. The method of claim 107, wherein the oligonucleotide is an antisense molecule.~~

~~109. The method of claim 108, wherein the oligonucleotide is a siRNA.~~

~~110. The method of claim 108, wherein the oligonucleotide is a shRNA.~~

~~111. The method of claims 107 through 110, wherein the oligonucleotide is between about 10 to about 30 nucleotides in length.~~

~~112. The method of claim 106, wherein the payload is an mRNA.~~

~~113. The method of claim 112, wherein the size of mRNA is about 1 kb to about 2 kb.~~

~~114. The method of claim 106, wherein the payload is a polypeptide.~~

~~115. The method of claim 114, wherein said polypeptide is between about 1,000 Da and about 10,000 Da.~~

~~116. The method of claim 106, wherein the payload is a small molecule.~~

~~117. The method of claim 116, wherein the small molecule is between about 100 Da and 1000 Da.~~

~~118. The method of claim 106, wherein the payload is dissolved in the first solution.~~

~~119. The method of claim 106, wherein the payload is dissolved in the second solution.~~

~~120. The method of claim 106, wherein the first solution is an aqueous buffer.~~

~~121. The method of claim 106, wherein the first solution comprises pH- and osmolality-controlled buffers.~~

~~122. The method of claim 106, wherein the organic phase of the second solution comprises methanol.~~

~~123. The method of claim 106, wherein the organic phase of the second solution comprises ethanol.~~

~~124. The method of claim 106, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.~~

~~125. The method of claim 124, wherein the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species.~~

~~126. The method of claim 124 or 125, wherein said second solution comprises more than one type of lipid.~~

~~127. The method of claim 124 or 125, wherein the total concentration of lipid is varied.~~

~~128. The method of claim 127, wherein the total concentration of lipid is varied between about 0.4 and about 4 mM.~~

~~129. The method of claim 124 or 125, wherein the percentage of lipids that are PEGylated is varied.~~

~~130. The method of claim 129, wherein the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.~~

~~131. The method of any one of claims 107-113, wherein the payload's N:P ratio is varied.~~

~~132. The method of claim 131, wherein the N:P ratio is varied between about 0.5 to about 5.~~

~~133. The method of any one of claims 106-132, wherein the LNP is a polymer lipid nanoparticle.~~

~~134. The method of claims 106-132, wherein the LNP is a liposome.~~

~~135. The method of claims 106-132, wherein the LNP is a lipoprotein nanoparticle.~~

~~136. The method of claim 106, wherein said first solution is injected into said second solution.~~

~~137. The method of claim 106, wherein said second solution is injected into said first solution.~~

~~138. The method of any one of claims 106-137, wherein the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%.~~

~~139. The method of any one of claims 106-137, wherein the optimal parameters are those which produce a LNP with a mean diameter of 80-200 nm, having an unimodal size distribution, and a polydispersity of less than about 30%.~~

~~140. The method of any one of claims 106-137, wherein the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.~~

~~141. 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.

142. The method of claim 141, wherein the payload is an oligonucleotide.

143. The method of claim 142, wherein the oligonucleotide is an antisense molecule.

144. The method of claim 142, wherein the oligonucleotide is a siRNA.

145. The method of claim 142, wherein the oligonucleotide is a shRNA.

146. The method of claims 142 through 145, wherein the oligonucleotide is between about 10 to about 30 nucleotides in length.

147. The method of claim 141, wherein the payload is an mRNA.

148. The method of claim 147, wherein the size of mRNA is about 1 kb to about 2 kb.

149. The method of claim 141, wherein the payload is a polypeptide.

150. The method of claim 149, wherein said polypeptide is between about 1,000 Da and about 10,000 Da.

151. The method of claim 141, wherein the payload is a small molecule.

152. The method of claim 151, wherein the small molecule is between about 100 Da and 1000 Da.

153. The method of claim 141, wherein the payload is dissolved in the first solution.

154. The method of claim 141, wherein the payload is dissolved in the second solution.

155. The method of claim 141, wherein the first solution is an aqueous buffer.

~~156. The method of claim 141, wherein the first solution comprises pH- and osmolality-controlled buffers.~~

~~157. The method of claim 141, wherein the organic phase of the second solution comprises methanol.~~

~~158. The method of claim 141, wherein the organic phase of the second solution comprises ethanol.~~

~~159. The method of claim 141, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.~~

~~160. The method of claim 159, wherein the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species.~~

~~161. The method of claim 159 or 160, wherein said second solution comprises more than one type of lipid.~~

~~162. The method of claim 159 or 160, wherein the total concentration of lipid is varied.~~

~~163. The method of claim 162, wherein the total concentration of lipid is varied between about 0.4 and about 4 mM.~~

~~164. The method of claim 159 or 160, wherein the percentage of lipids that are PEGylated is varied.~~

~~165. The method of claim 164, wherein the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.~~

~~166. The method of any one of claims 142-150, wherein the payload's N:P ratio is varied.~~

~~167. The method of claim 166, wherein the N:P ratio is varied between about 0.5 to about 5.~~

~~168. The method of any one of claims 141-167, wherein the LNP is a polymer lipid nanoparticle.~~

~~169. The method of claims 141-167, wherein the LNP is a liposome.~~

~~170. The method of claims 141-167, wherein the LNP is a lipoprotein nanoparticle.~~

~~171. The method of claim 141, wherein said first solution is injected into said second solution.~~

~~172. The method of claim 141, wherein said second solution is injected into said first solution.~~

~~173. The method of any one of claims 141-172, wherein the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%.~~

~~174. The method of any one of claims 141-172, wherein the optimal parameters are those which produce a LNP with a mean diameter of 80-200 nm, having an unimodal size distribution, and a polydispersity of less than about 30%.~~

~~175. The method of any one of claims 141-172, wherein the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.~~

~~176~~23. An optimized lipid nanoparticle (LNP) preparation manufactured by ~~a~~ the process ~~comprising the following steps~~of any one of claims 1-21.~~:~~

~~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.~~

~~177. The method of claim 176, wherein the payload is an oligonucleotide.~~

~~178. The method of claim 177, wherein the oligonucleotide is an antisense molecule.~~

~~179. The method of claim 178, wherein the oligonucleotide is a siRNA.~~

~~180. The method of claim 178, wherein the oligonucleotide is a shRNA.~~

~~181. The method of claims 177 through 180, wherein the oligonucleotide is between about 10 to about 30 nucleotides in length.~~

~~182. The method of claim 176, wherein the payload is an mRNA.~~

~~183. The method of claim 182, wherein the size of mRNA is about 1 kb to about 2 kb.~~

~~184. The method of claim 176, wherein the payload is a polypeptide.~~

~~185. The method of claim 184, wherein said polypeptide is between about 1,000 Da and about 10,000 Da.~~

~~186. The method of claim 176, wherein the payload is a small molecule.~~

~~187. The method of claim 186, wherein the small molecule is between about 100 Da and 1000 Da.~~

~~188. The method of claim 176, wherein the payload is dissolved in the first solution.~~

~~189. The method of claim 176, wherein the payload is dissolved in the second solution.~~

~~190. The method of claim 176, wherein the first solution is an aqueous buffer.~~

~~191. The method of claim 176, wherein the first solution comprises pH- and osmolality-controlled buffers.~~

~~192. The method of claim 176, wherein the organic phase of the second solution comprises methanol.~~

~~193. The method of claim 176, wherein the organic phase of the second solution comprises ethanol.~~

~~194. The method of claim 176, wherein the self-assembling molecules include at least a lipid component comprised of at least one species of lipid molecule.~~

~~195. The method of claim 194, wherein the at least one species of lipid molecule is selected from a cationic lipid species, a non-cationic lipid species, and a phospholipid species.~~

~~196. The method of claim 194 or 195, wherein said second solution comprises more than one type of lipid.~~

~~197. The method of claim 194 or 195, wherein the total concentration of lipid is varied.~~

~~198. The method of claim 197, wherein the total concentration of lipid is varied between about 0.4 and about 4 mM.~~

~~199. The method of claim 194 or 195, wherein the percentage of lipids that are PEGylated is varied.~~

~~200. The method of claim 199, wherein the percentage of lipids that are PEGylated are varied between about 0.5% to about 5% of the total lipid composition.~~

~~201. The method of any one of claims 177-183, wherein the payload's N:P ratio is varied.~~

~~202. The method of claim 201, wherein the N:P ratio is varied between about 0.5 to about 5.~~

~~203~~24. The LNP preparation~~method~~ of ~~any one of~~ claim~~s 176-202~~22 or 23, wherein the LNP preparation comprises~~is a~~ polymer lipid nanoparticles, liposomes, or lipoprotein nanoparticles.

~~204. The method of claims 176-202, wherein the LNP is a liposome.~~

~~205. The method of claims 176-202, wherein the LNP is a lipoprotein nanoparticle.~~

~~206. The method of claim 176, wherein said first solution is injected into said second solution.~~

~~207. The method of claim 176, wherein said second solution is injected into said first solution.~~

~~208. The method of any one of claims 176-207, wherein the optimal parameters are those which produce an encapsulation efficiency of the payload greater than 80%.~~

~~209. The method of any one of claims 176-207, wherein the optimal parameters are those which produce a LNP with a mean diameter of 80-200 nm, having an unimodal size distribution, and a polydispersity of less than about 30%.~~

~~210~~25. The optimized LNP preparation~~method~~ of ~~any one of~~ claim~~s~~ 23~~176-207~~, wherein the LNPs maintain a similar size distribution and payload encapsulation for at least one month under storage in solution at 4 degrees Celsius.

~~211~~26. A workflow for HTS screening of a plurality of parameters for LNP formation, comprising:

(i) a robotic liquid handler;

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

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

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

wherein said parameters are systematically varied between microwells; and

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

~~212. The method of claim 211, wherein 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.~~

~~213. The method of claim 211, wherein said desired LNP characteristics are selected from the group consisting of: average particle size, particle size distribution, encapsulation efficiency, and particle stability.~~

~~214. The workflow of claim 211, wherein said instrument is capable of either dynamic light scattering (DLS), ultraviolet-visible (UV-Vis), or fluorescence spectroscopy.~~