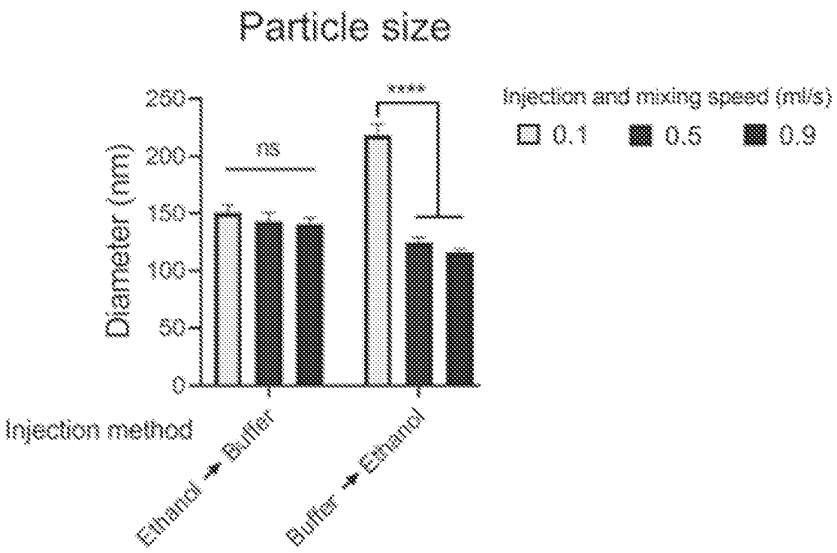

Particle size
Diameter (nm)
250
200
150
100
50
0
ns
****
Injection and mixing speed (ml/s)
0.1
0.5
0.9
Injection method
Ethanol → Buffer
Buffer → Ethanol

FIG. 1A

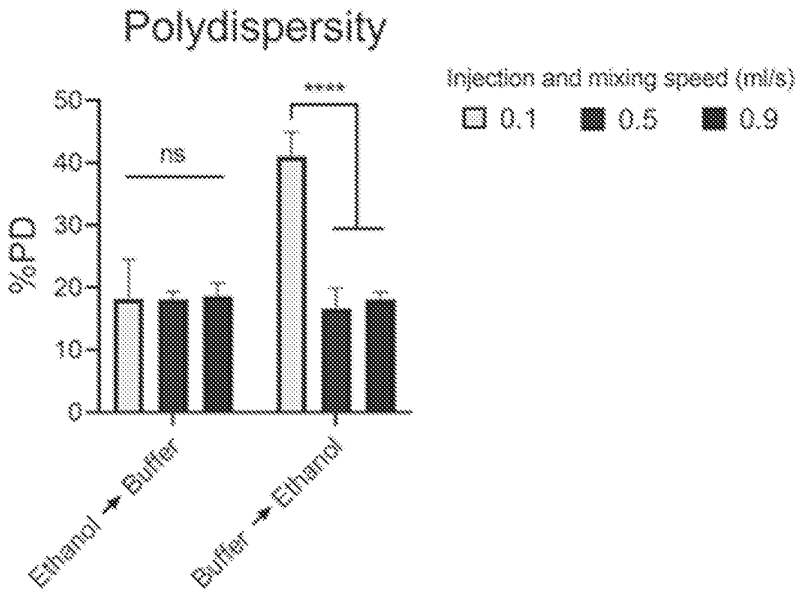

Polydispersity
%PD
50
40
30
20
10
0
ns
****
Injection and mixing speed (ml/s)
0.1
0.5
0.9
Ethanol → Buffer
Buffer → Ethanol

FIG. 1B

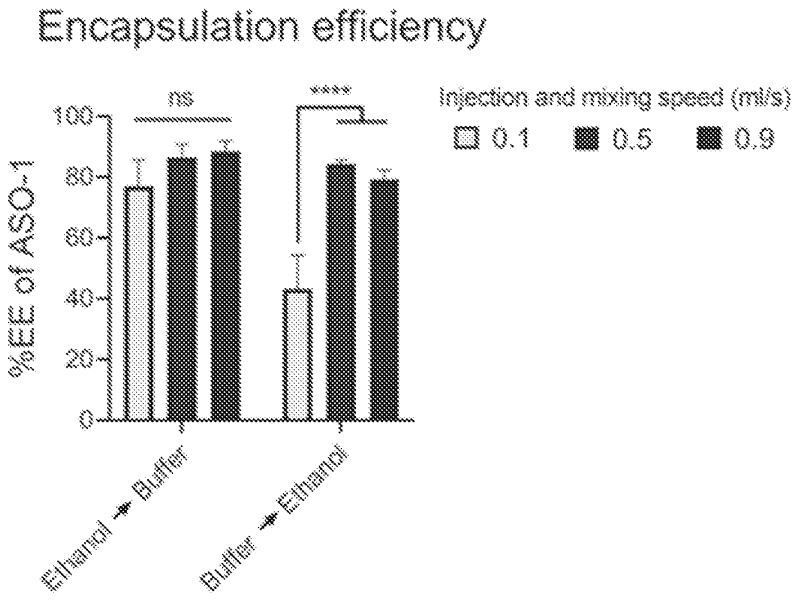

Encapsulation efficiency
ns
****
Injection and mixing speed (ml/s)
0.1
0.5
0.9
%EE of ASO-1
100
80
60
40
20
0
Ethanol → Buffer
Buffer → Ethanol

FIG. 1C

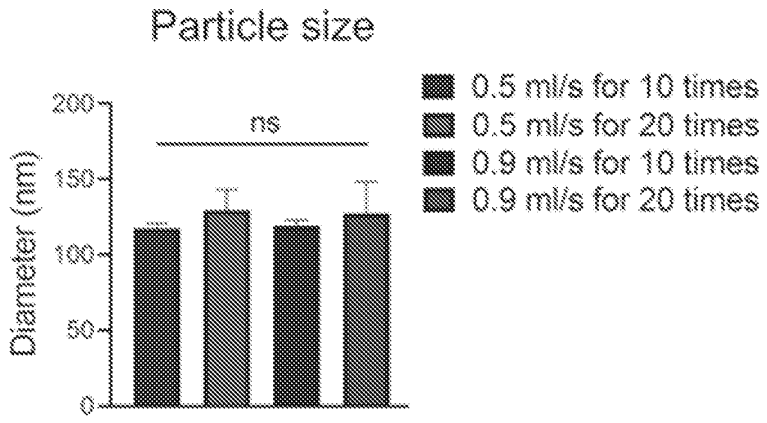

Particle size
0.5 ml/s for 10 times
0.5 ml/s for 20 times
0.9 ml/s for 10 times
0.9 ml/s for 20 times
ns
Diameter (nm)
200
150
100
50
0

FIG. 1D

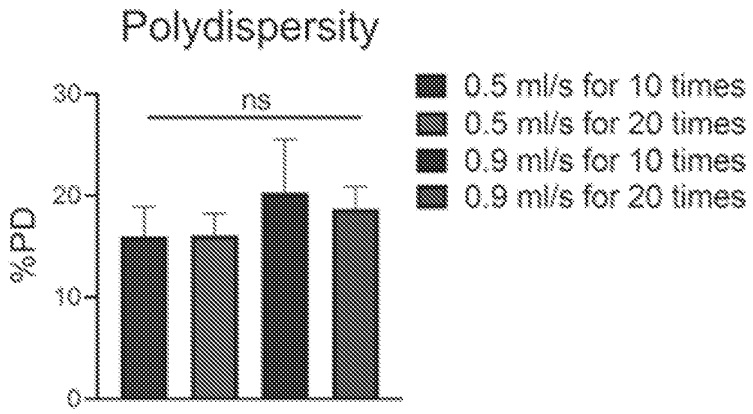

Polydispersity
ns
%PD
30
20
10
0
0.5 ml/s for 10 times
0.5 ml/s for 20 times
0.9 ml/s for 10 times
0.9 ml/s for 20 times

FIG. 1E

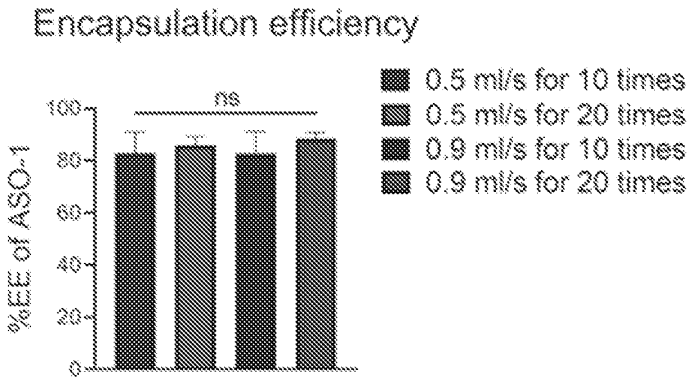

Encapsulation efficiency
ns
%EE of ASO-1
100
80
60
40
20
0
0.5 ml/s for 10 times
0.5 ml/s for 20 times
0.9 ml/s for 10 times
0.9 ml/s for 20 times

FIG. 1F

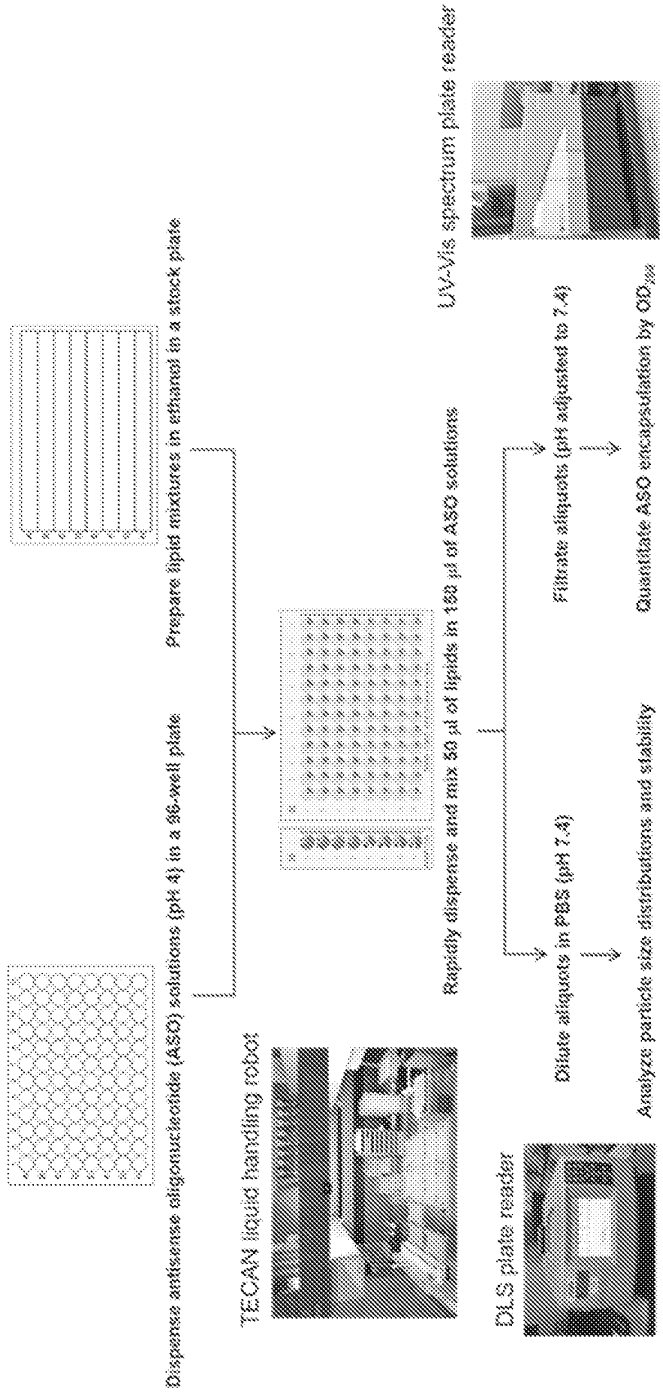

Dispense antisense oligonucleotide (ASO) solutions (pH 4) in a 96-well plate
Prepare lipid mixtures in ethanol in a stock plate
TECAN liquid handling robot
Rapidly dispense and mix 50 µl of lipids in 150 µl of ASO solutions
UV-Vis spectrum plate reader
Dilute aliquots in PBS (pH 7.4)
Filtrate aliquots (pH adjusted to 7.4)
DLS plate reader
Analyze particle size distributions and stability
Quantitate ASO encapsulation by OD_{260}
Determine the optimal lipid nanoparticle formulations with uniform, stable particle size and high ASO loading
Lipid composition: molar ratio of charged lipid / DSPC / cholesterol / DSPE-PEG_{2000} = 50 / 10 / 38.5-x / x, x = 0, 1.5, 3, or 5
Lipid amount: final total lipid concentration = 1 or 2 mM / well
ASO loading levels: N/P ratio = 8, 2, 1, or 0.5

FIG. 2

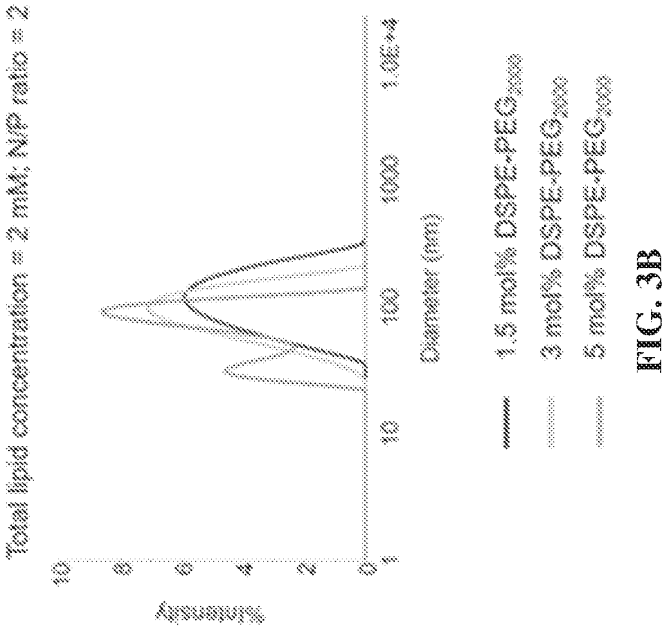

Total lipid concentration = 2 mM; N/P ratio = 2
Intensity
Diameter (nm)
1.5 mol% DSPE-PEG2000
3 mol% DSPE-PEG2000
5 mol% DSPE-PEG2000

FIG. 3B

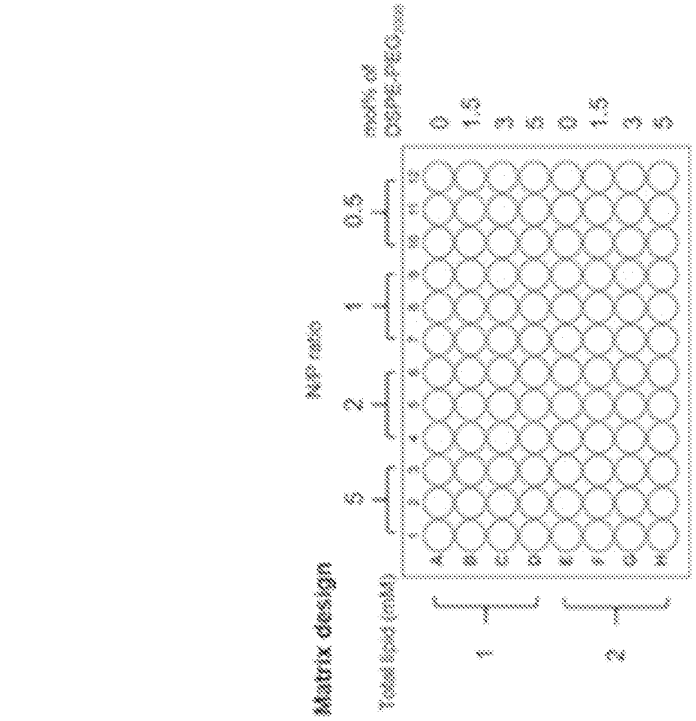

Matrix design
Total lipid (mM)
N/P ratio
mol% of DSPE-PEG2000

FIG. 3A

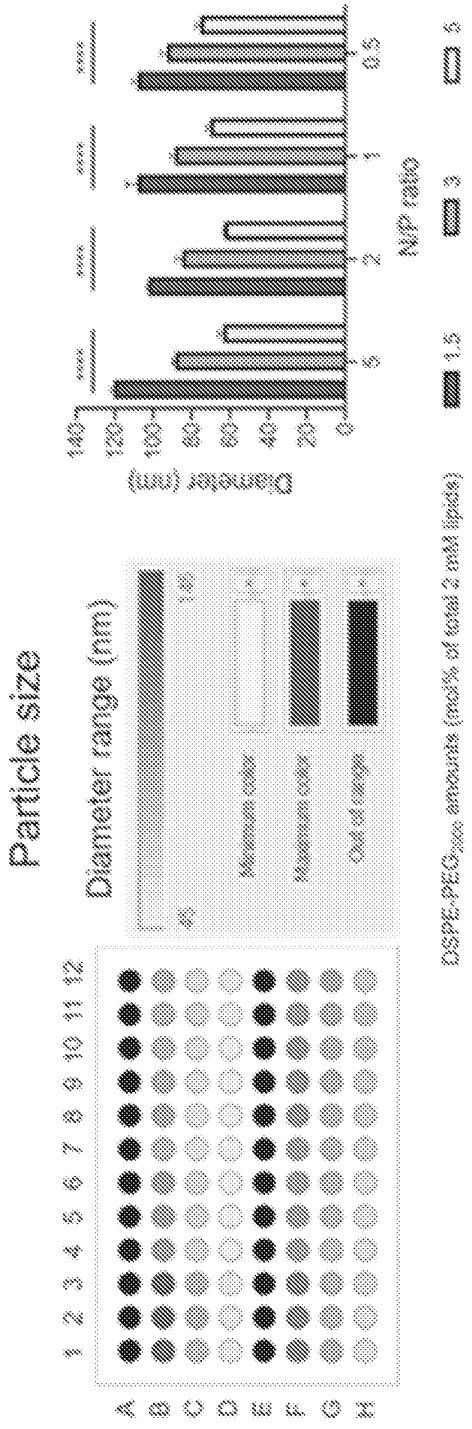

Particle size
Diameter range (nm)
40
100
Minimum value
Minimum value
Out of range
1
2
3
4
5
6
7
8
9
10
11
12
A
B
C
D
E
F
G
H
Diameter (nm)
140
120
100
80
60
40
20
0
0.5
1
2
3
5
N/P ratio
1.5
3
5
DSPE-PEG2000 amounts (mol% of total 2 mM lipids)

FIG. 3C

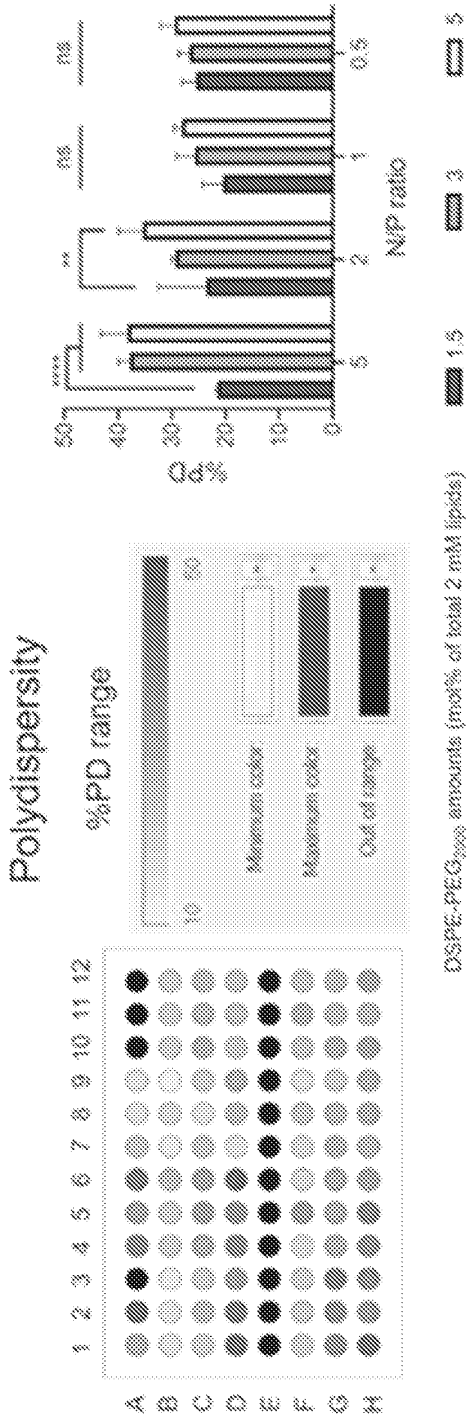

Polydispersity
%PD range
10
30
Minimum color
Maximum color
Out of range
A B C D E F G H
1 2 3 4 5 6 7 8 9 10 11 12
Qd%
50
40
30
20
10
0
ns
ns
ns
N/P ratio
5
2
1
0.5
DSPE-PEG2000 amounts (mol% of total 2 mM lipids)
1.5
3
5

FIG. 3D

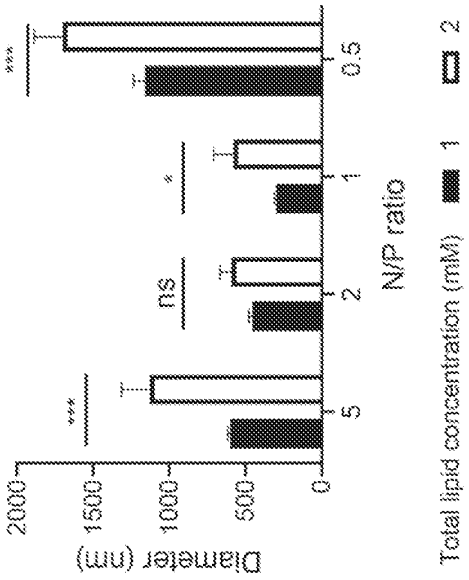

Diameter (nm)
2000
1500
1000
500
0
***
ns
*
***
5
2
1
0.5
N/P ratio
Total lipid concentration (mM)
1
2

FIG. 4

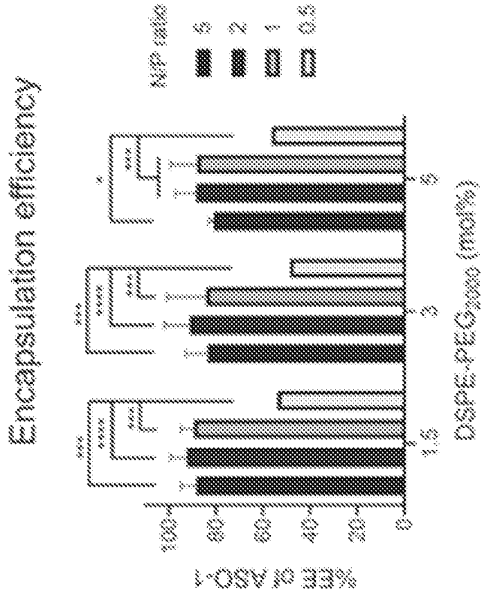

Encapsulation efficiency
%EE of ASO-1
100
80
60
40
20
0
****
****
*
1.5
3
6
DSPE-PEG2000 (mol%)
N/P ratio
6
2
1
0.5

FIG. 3E

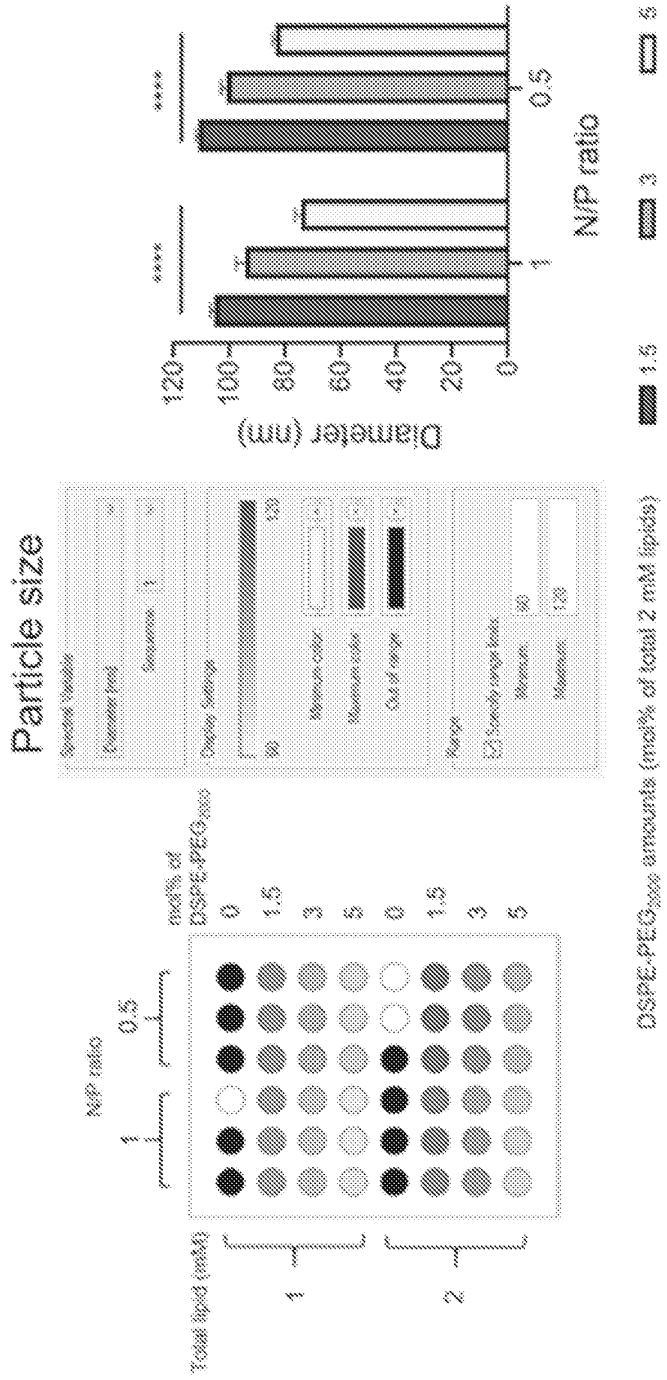

Particle size
Diameter (nm)
N/P ratio
1.5
3
5
DSPE-PEG2000 amounts (mol% of total 2 mM lipids)
Total lipid (mM)
mol% of DSPE-PEG2000

FIG. 5A

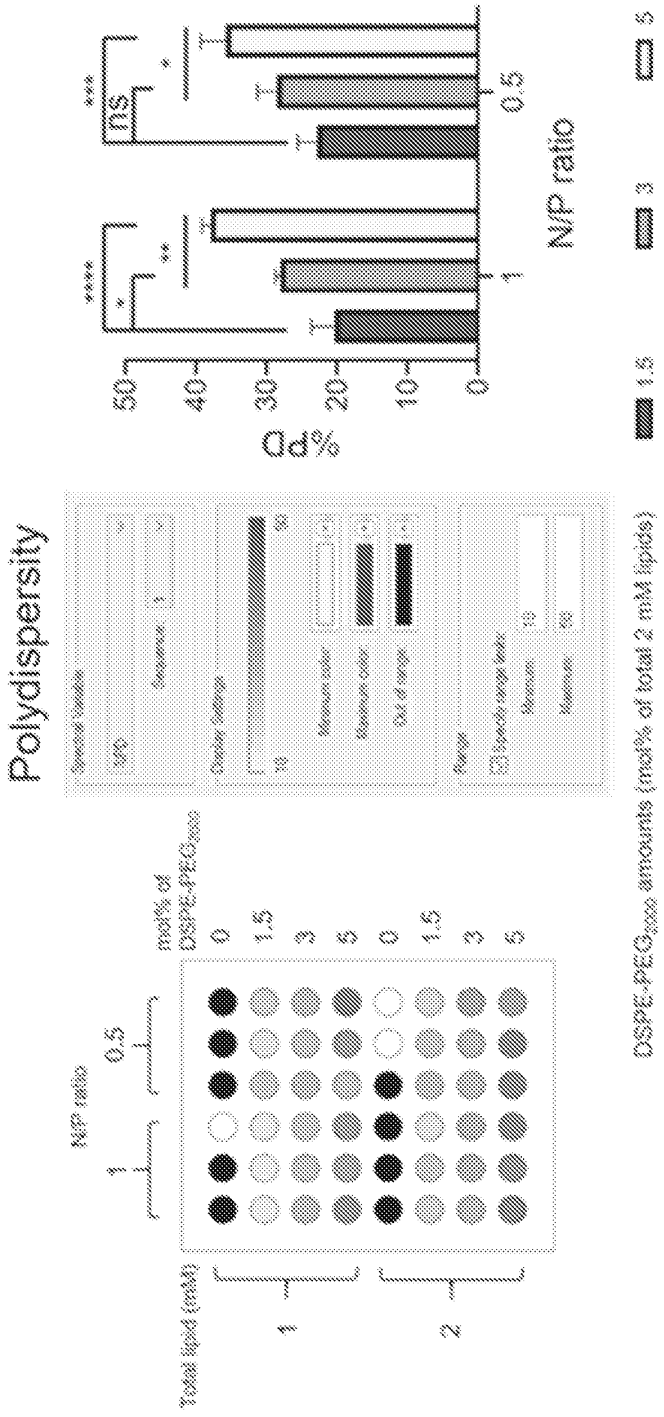

PDI%
50
40
30
20
10
0
1
0.5
N/P ratio
1.5
3
5
Polydispersity
Total lipid (mM)
N/P ratio
mol% of DSPE-PEG2000
DSPE-PEG2000 amounts (mol% of total 2 mM lipids)

FIG. 5B

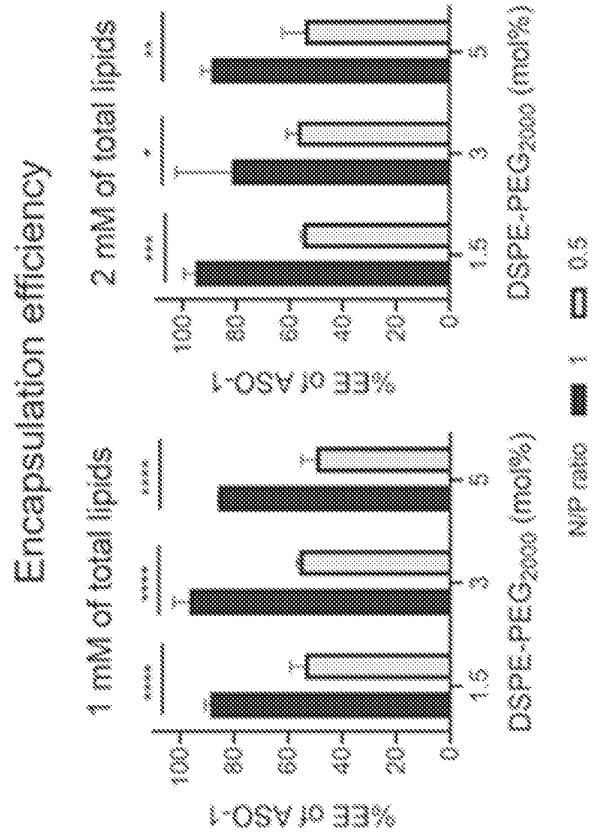

Encapsulation efficiency
1 mM of total lipids
2 mM of total lipids
%EE of ASO-1
100
80
60
40
20
0
****
****
****
***
*
**
1
1.5
3
5
DSPE-PEG2000 (mol%)
N/P ratio
1
0.5

FIG. 5C

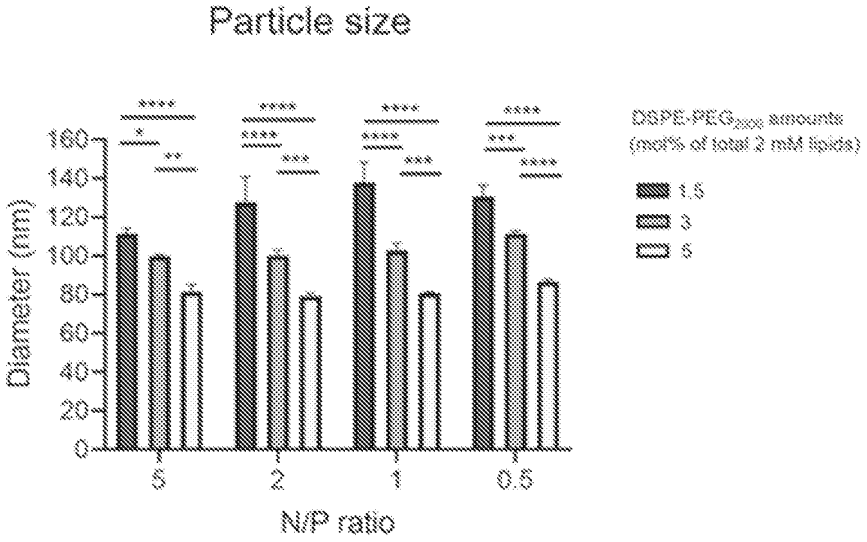

Particle size
Diameter (nm)
160
140
120
100
80
60
40
20
0
5
2
1
0.5
N/P ratio
DSPE-PEG2000 amounts
(mol% of total 2 mM lipids)
1.5
3
5

FIG. 6A

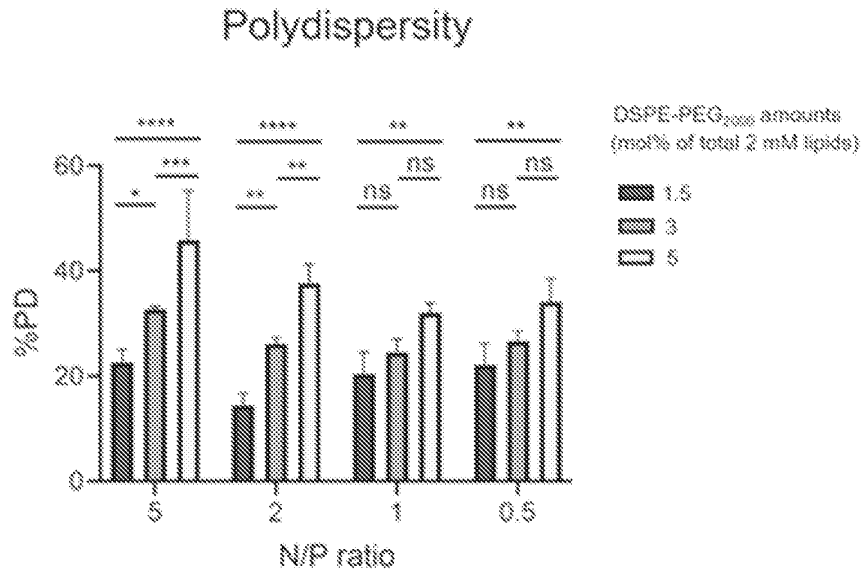

Polydispersity
%PD
60
40
20
0
5
2
1
0.5
N/P ratio
DSPE-PEG2000 amounts
(mol% of total 2 mM lipids)
1.5
3
5

FIG. 6B

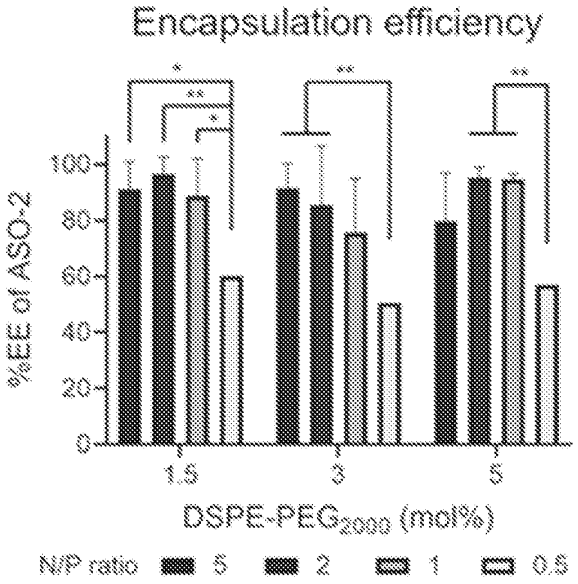

Encapsulation efficiency
%EE of ASO-2
100
80
60
40
20
0
1.5
3
5
DSPE-PEG2000 (mol%)
N/P ratio
5
2
1
0.5

FIG. 6C

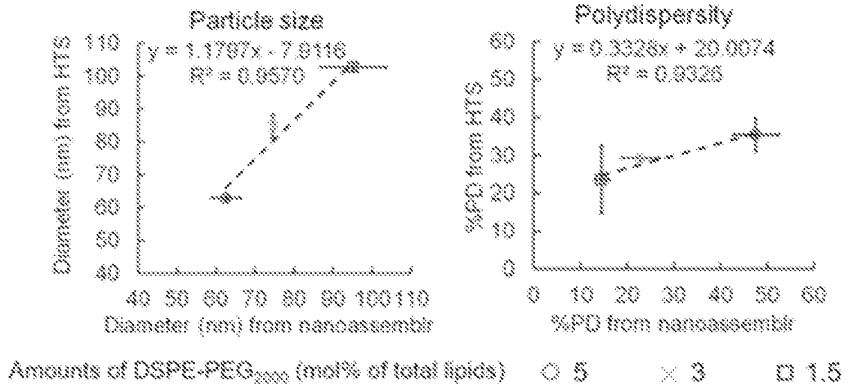

Particle size
y = 1.1797x - 7.9116
R² = 0.9570
Diameter (nm) from HTS
Diameter (nm) from nanoassembl
Polydispersity
y = 0.3328x + 20.0074
R² = 0.9328
%PD from HTS
%PD from nanoassembl
Amounts of DSPE-PEG2000 (mol% of total lipids)
5
3
1.5

FIG. 7A

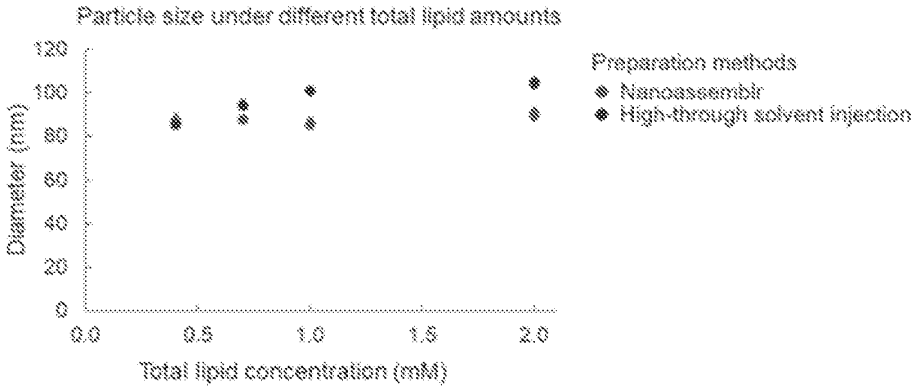


FIG. 7B

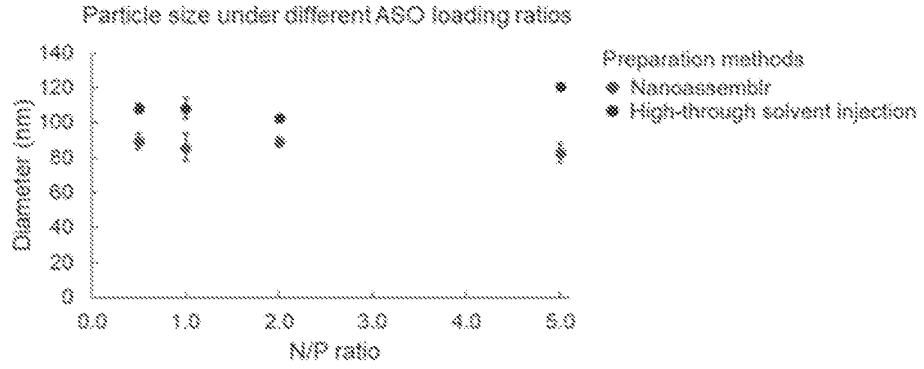


FIG. 7C

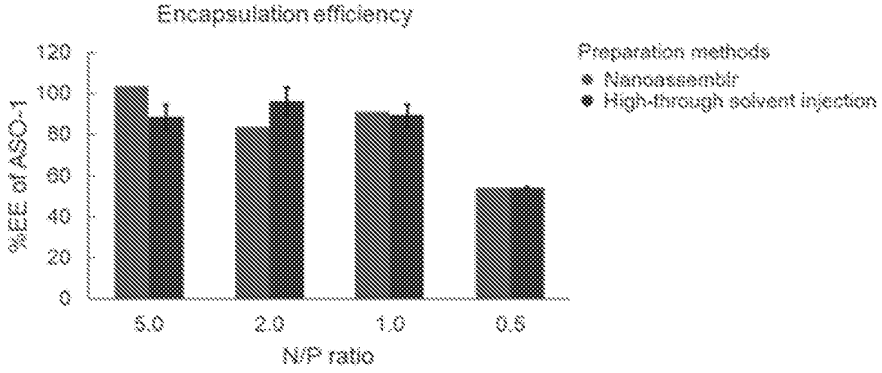


FIG. 7D

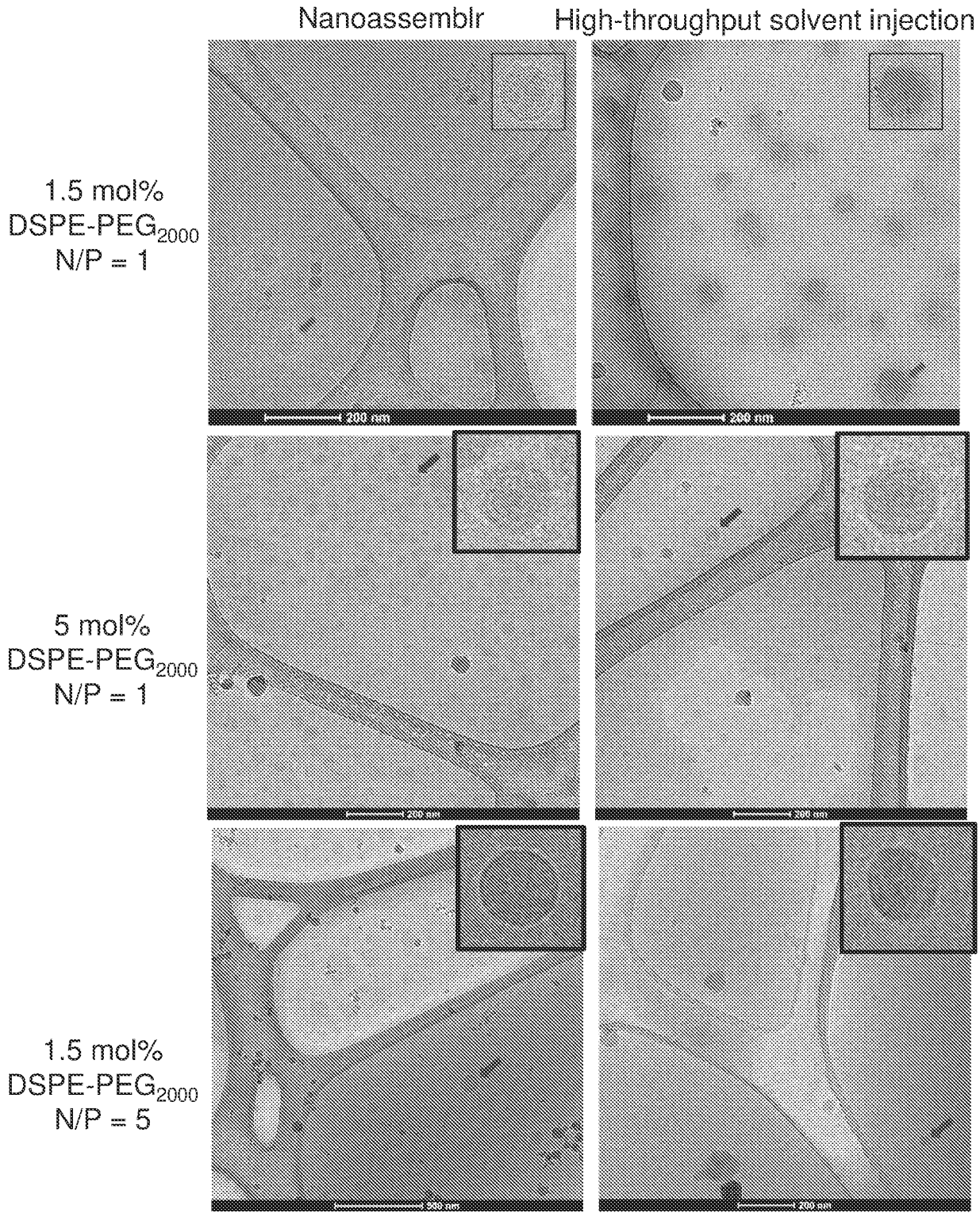

Nanoassemblr
High-throughput solvent injection
1.5 mol% DSPE-PEG2000 N/P = 1
5 mol% DSPE-PEG2000 N/P = 1
1.5 mol% DSPE-PEG2000 N/P = 5
200 nm

FIG. 7E

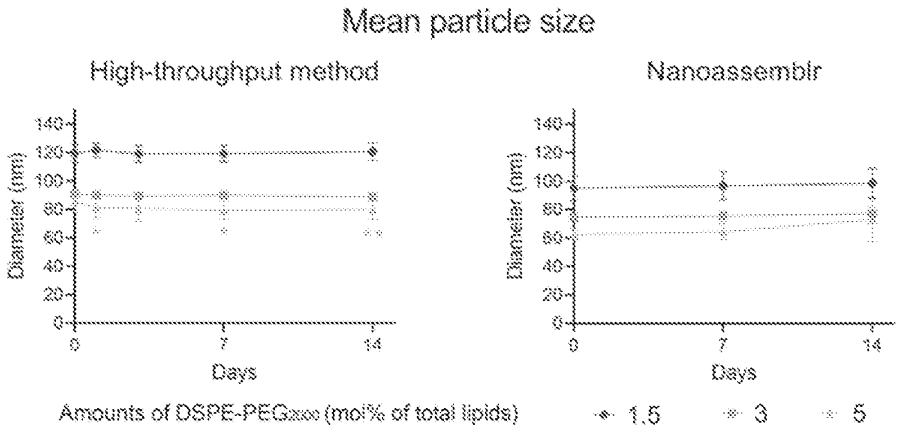

Mean particle size
High-throughput method
Nanoassemblr
Diameter (nm)
Days
Amounts of DSPE-PEG2000 (mol% of total lipids)
1.5
3
5

FIG. 8A

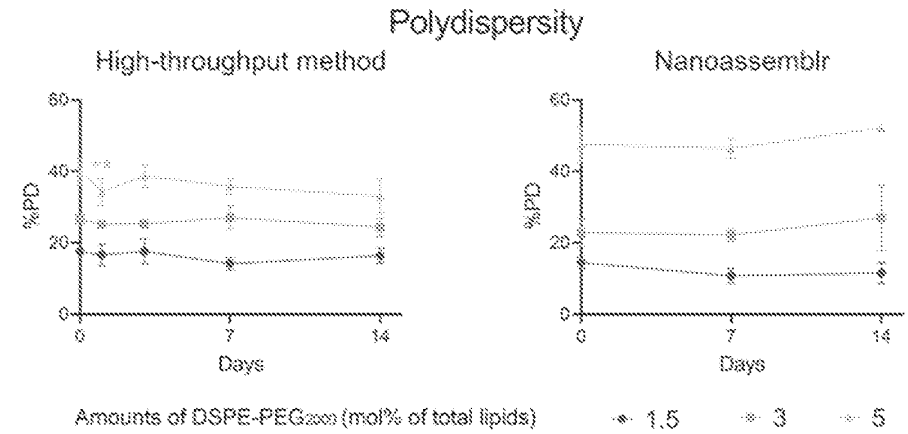

Polydispersity
High-throughput method
Nanoassemblr
%PD
Days
Amounts of DSPE-PEG2000 (mol% of total lipids)
1.5
3
5

FIG. 8B

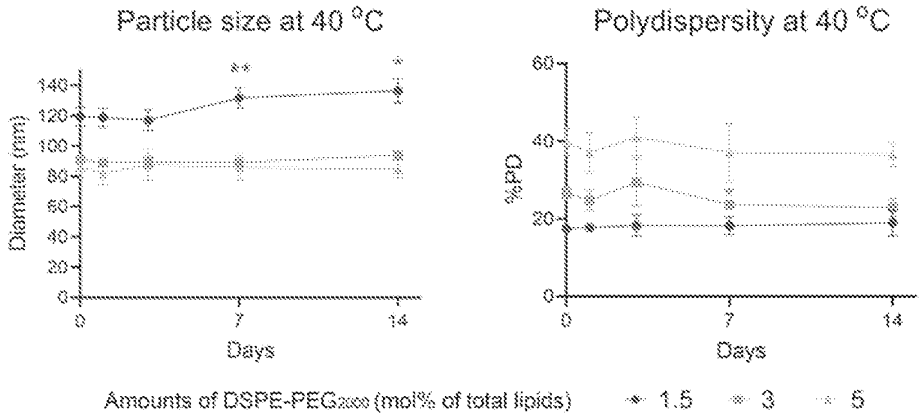

Particle size at 40 °C
Diameter (nm)
Days
Polydispersity at 40 °C
PDI
Days
Amounts of DSPE-PEG2000 (mol% of total lipids)
1.5
3
5

FIG. 9

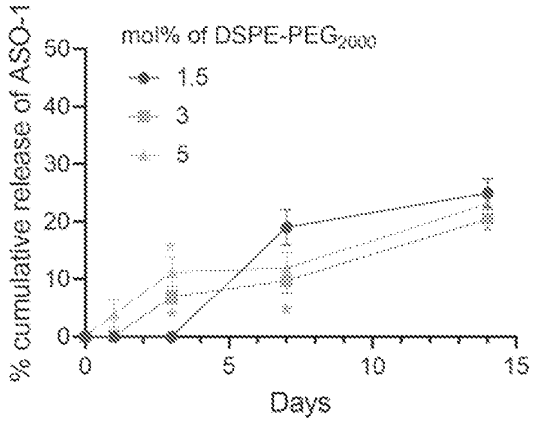

% cumulative release of ASO-1
mol% of DSPE-PEG2000
1.5
3
5
Days

FIG. 10

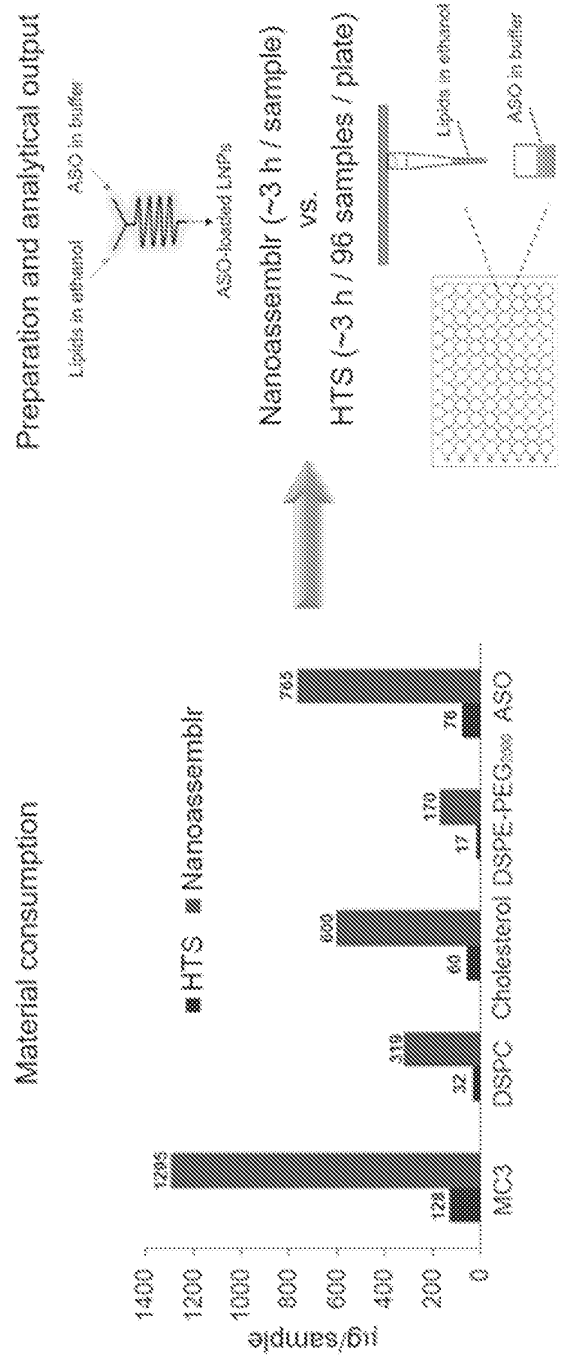

Material consumption
HTS
Nanoassembir
μg/sample
1400
1200
1000
800
600
400
200
0
1295
128
MC3
115
32
DSPC
600
60
Cholesterol
178
17
DSPE-PEG2000
765
76
ASO
Preparation and analytical output
Lipids in ethanol
ASO in buffer
ASO-loaded LNPs
Nanoassembir (~3 h / sample)
vs.
HTS (~3 h / 96 samples / plate)
Lipids in ethanol
ASO in buffer

FIG. 11

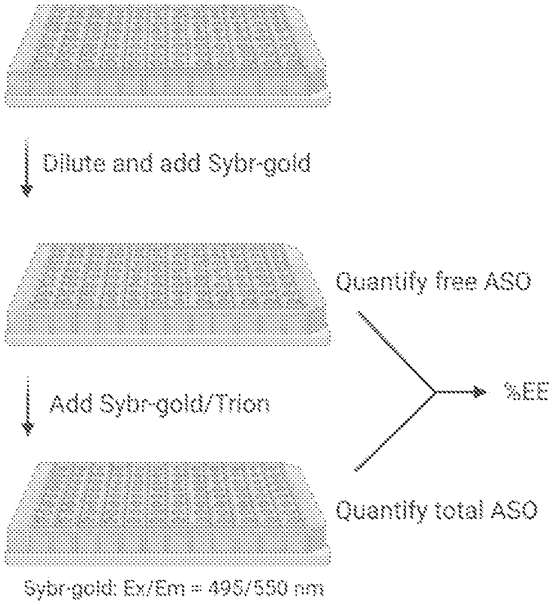


FIG. 12A

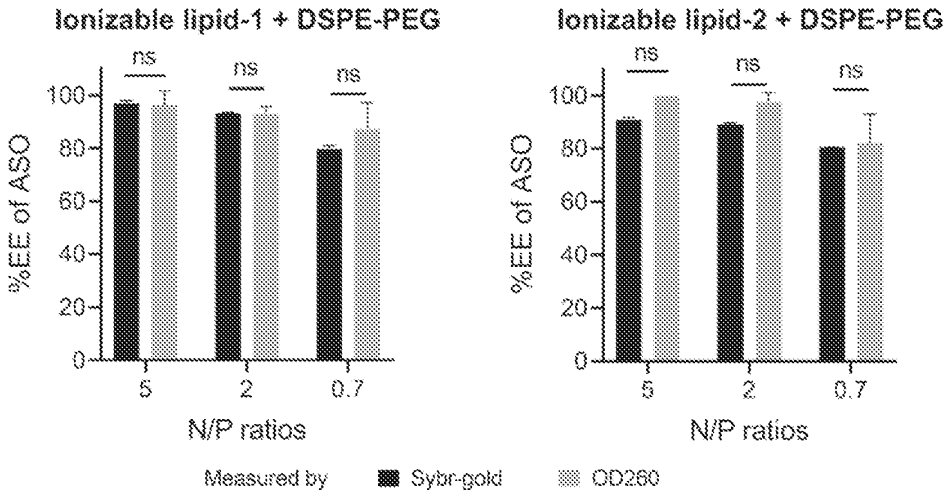


FIG. 12B

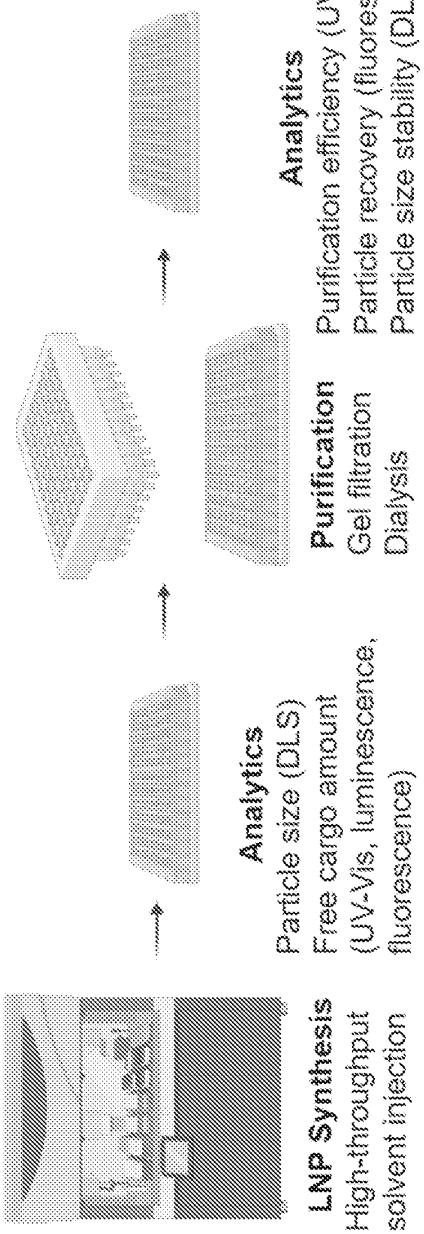

LNP Synthesis
High-throughput solvent injection
Analytics
Particle size (DLS)
Free cargo amount (UV-Vis, luminescence, fluorescence)
Purification
Gel filtration
Dialysis
Analytics
Purification efficiency (UV-Vis)
Particle recovery (fluorescence)
Particle size stability (DLS)

FIG. 13A

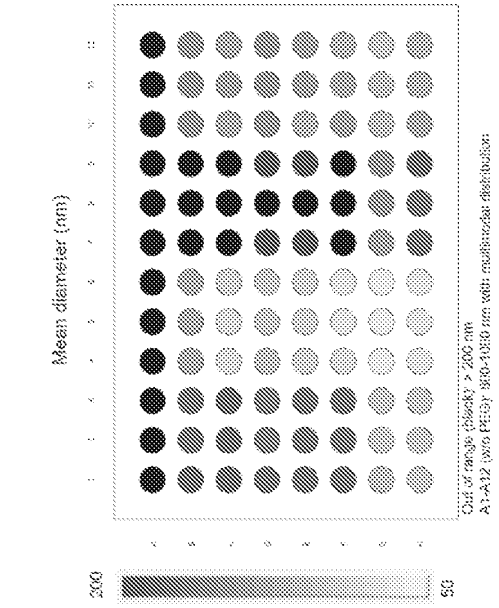


FIG. 13C

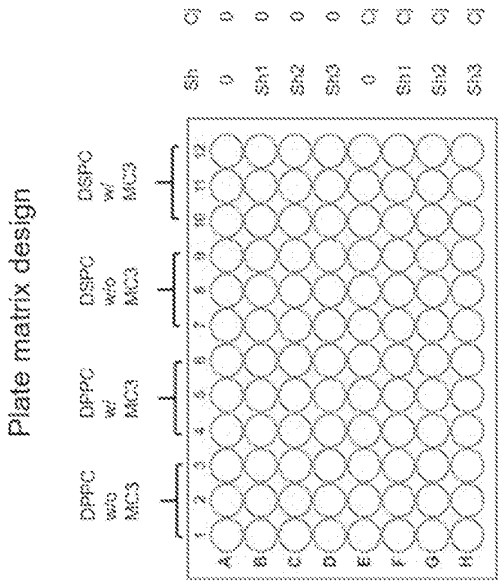


FIG. 13B

Free peptide concentration before purification

Free HIBIT conc. (mg/ml)	1	2	3	4	5	6	7	8	9	10	11	12
A	1.35	1.42	1.43	1.43	1.41	1.41	1.46	1.42	1.47	1.48	1.40	1.48
B	1.07	1.39	1.35	1.36	1.34	1.33	1.31	1.31	1.29	1.34	1.27	1.76
C	1.51	1.33	1.36	1.41	1.34	1.30	1.34	1.36	1.23	1.42	1.40	1.35
D	1.13	1.31	1.36	1.37	1.32	1.30	1.32	1.30	1.29	1.31	1.28	1.55
E	1.60	1.35	1.34	1.38	1.38	1.32	1.30	1.35	1.31	1.33	1.32	1.26
F	1.09	1.37	1.36	1.48	1.41	1.39	1.32	1.35	1.34	1.39	1.36	1.74
G	1.58	1.38	1.37	1.33	1.39	1.52	1.32	1.32	1.42	1.36	1.39	1.34
H	1.40	1.37	1.45	1.40	1.42	1.37	1.40	1.38	1.50	1.45	1.36	1.67

FIG. 13D

Free peptide concentration after gel filtration

HIBIT conc. (mg/ml) after SEC	1	2	3	4	5	6	7	8	9	10	11	12
A	0.027	0.029	0.030	0.027	0.032	0.034	0.026	0.030	0.037	0.021	0.028	0.030
B	0.033	0.031	0.033	0.043	0.030	0.037	0.056	0.036	0.049	0.076	0.028	0.032
C	0.035	0.026	0.029	0.028	0.026	0.029	0.036	0.037	0.033	0.035	0.036	0.035
D	0.016	0.029	0.000	0.000	0.026	0.028	0.032	0.043	0.016	0.036	0.030	0.032
E	0.000	0.037	0.027	0.027	0.064	0.029	0.041	0.062	0.027	0.036	0.048	0.035
F	0.032	0.028	0.032	0.032	0.027	0.036	0.034	0.045	0.039	0.030	0.032	0.155
G	0.034	0.032	0.030	0.027	0.029	0.040	0.033	0.030	0.039	0.029	0.031	0.047
H	0.036	0.037	0.035	0.034	0.032	0.033	0.033	0.031	0.000	0.038	0.034	0.029

FIG. 13E

Free peptide concentration after dialysis

HBET conc. (mg/ml) after dialysis	1	2	3	4	5	6	7	8	9	10	11	12
A	0.093	0.098	0.140	0.113	0.101	0.115	0.121	0.101	0.121	0.111	0.112	0.116
B	0.106	0.104	0.087	0.100	0.117	0.102	0.104	0.094	0.112	0.093	0.098	0.103
C	0.099	0.070	0.093	0.108	0.105	0.111		0.102	0.075	0.094	0.095	0.105
D	0.096	0.101	0.059	0.102	0.094	0.110	0.108	0.100	0.104	0.113	0.054	0.103
E	0.091	0.106	0.098	0.104	0.105	0.110	0.115	0.103	0.103	0.107	0.099	0.103
F	0.064	0.093	0.096	0.109	0.100	0.101	0.102	0.091	0.104	0.099	0.093	0.104
G			0.118	0.091	0.107	0.114	0.105	0.107	0.111	0.117	0.121	0.119
H		0.106	0.100	0.116	0.114	0.124		0.104	0.112	0.116	0.109	0.111

FIG. 13F

Particle recovery rate after gel filtration

% Particle recovery after SEC	1	2	3	4	5	6	7	8	9	10	11	12
A	72.3	70.9	62.6	47.6	55.2	30.9	41.3	47.4	19.8	42.8	46.1	39.5
B	128.4	106.0	112.2	109.2	112.8	116.1	83.3	93.4	94.1	65.2	92.5	109.2
C	117.3	108.8	97.5	113.4	122.6	111.3	97.1	92.7	98.4	120.4	116.3	109.7
D	107.2	65.8	53.5	89.7	80.7	90.4	81.4	92.9	96.6	106.6	78.7	99.5
E	92.8	108.1	46.7	99.5	108.5	101.2	83.0	88.1	99.7	109.7	132.0	84.3
F	102.3	97.0	104.1	99.6	105.5	109.0	86.6	84.9	87.5	90.5	93.3	109.1
G	115.1	117.8	108.1	120.4	135.1	112.6	125.8	98.3	111.3	99.9	120.5	86.9
H	117.3	93.0	89.4	126.7	82.6	88.6	76.0	93.6	78.7	105.2	99.6	99.8

FIG. 13G

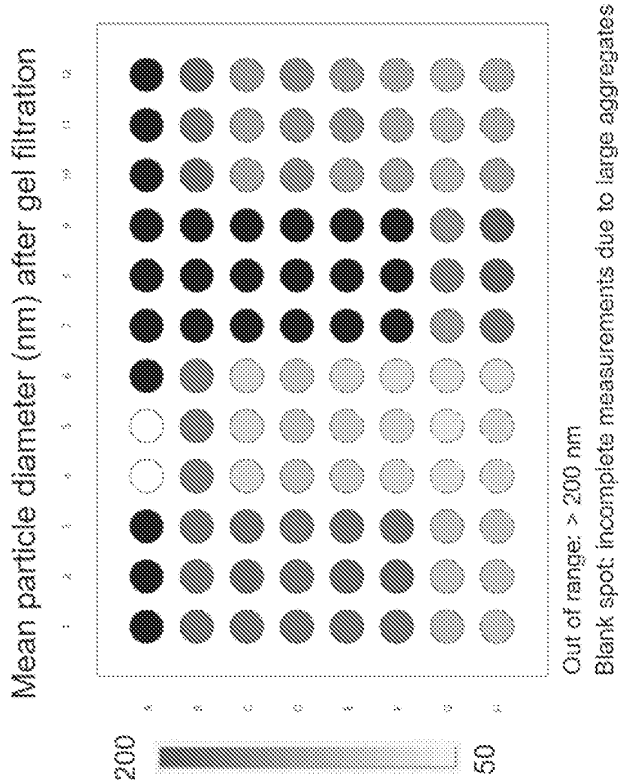

Mean particle diameter (nm) after gel filtration
200
50
Out of range: > 200 nm
Blank spot: incomplete measurements due to large aggregates

FIG. 13H