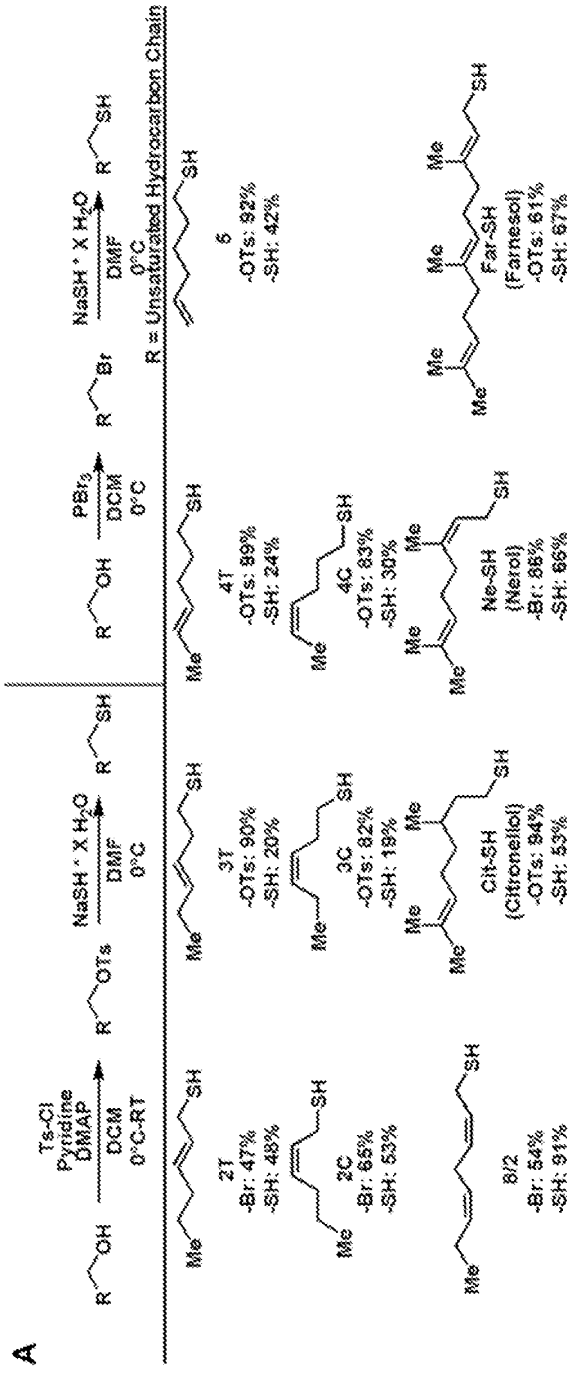

A
Ts-Cl
Pyridine
DMAP
DCM
0°C-RT
NaSH * X H2O
DMF
0°C
PBr3
DCM
0°C
NaSH * X H2O
DMF
0°C
R = Unsaturated Hydrocarbon Chain
2T
-Br: 47%
-SH: 48%
2C
-Br: 65%
-SH: 53%
8/2
-Br: 54%
-SH: 91%
3T
-OTs: 90%
-SH: 20%
3C
-OTs: 82%
-SH: 19%
Cit-SH
(Citronellol)
-OTs: 84%
-SH: 53%
4T
-OTs: 89%
-SH: 24%
4C
-OTs: 83%
-SH: 30%
Ne-SH
(Nerol)
-Br: 86%
-SH: 66%
6
-OTs: 92%
-SH: 42%
Far-SH
(Farnesol)
-OTs: 61%
-SH: 67%

FIG. 1A

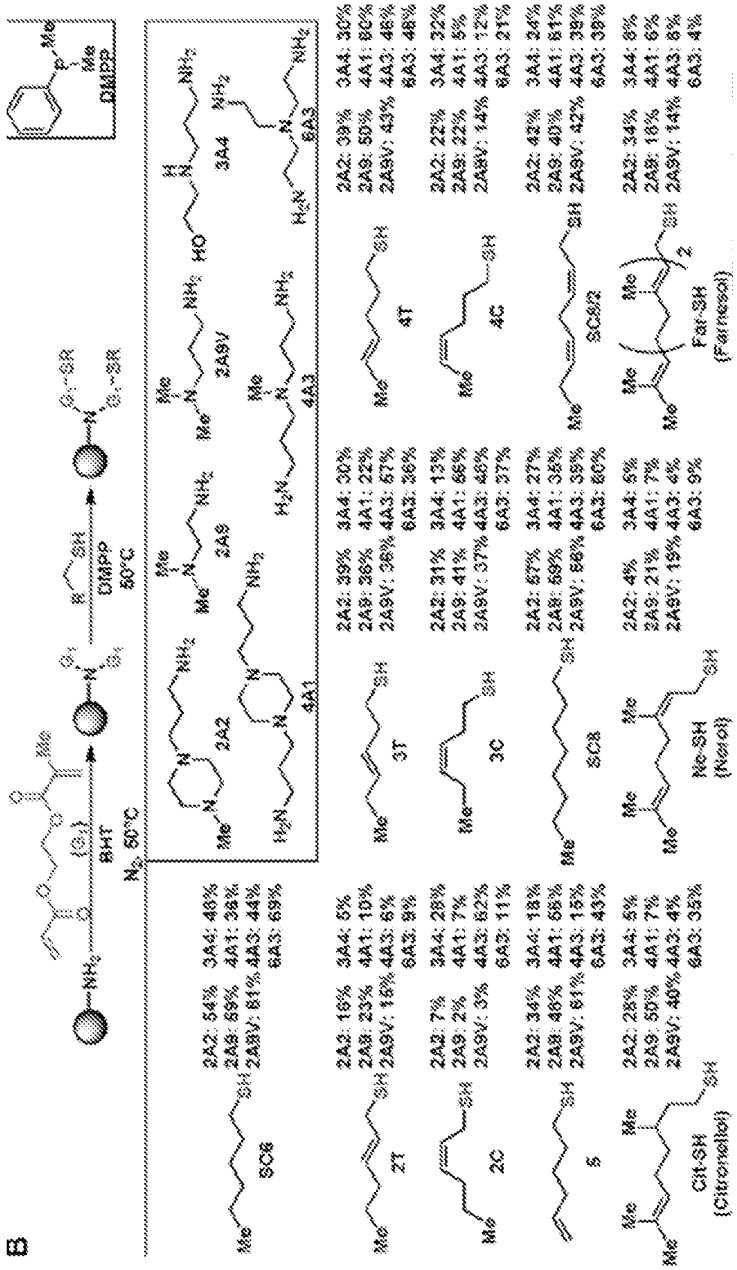

B
DMPP
80°C
BHT
N2, 60°C
2A2
2A9
2A9V
3A4
4A1
4A3
6A3
SC6
2T
2C
5
3T
3C
SC8
4T
4C
SC8/2
Cit-SH
(Citronellol)
Ne-SH
(Nerol)
Far-SH
(Farnesol)

FIG. 1B

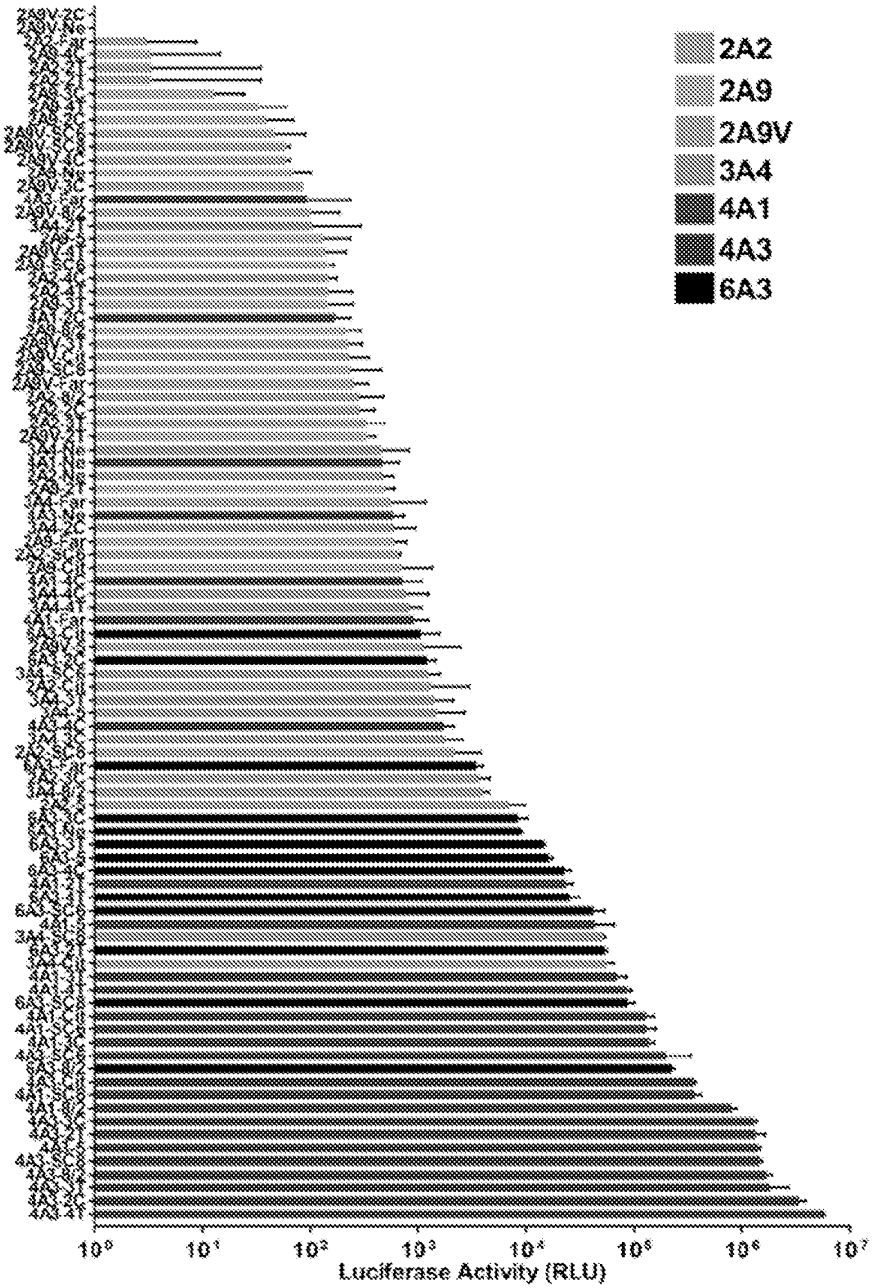

2A2
2A9
2A9V
3A4
4A1
4A3
6A3
10⁰
10¹
10²
10³
10⁴
10⁵
10⁶
10⁷
Luciferase Activity (RLU)

FIG. 2A

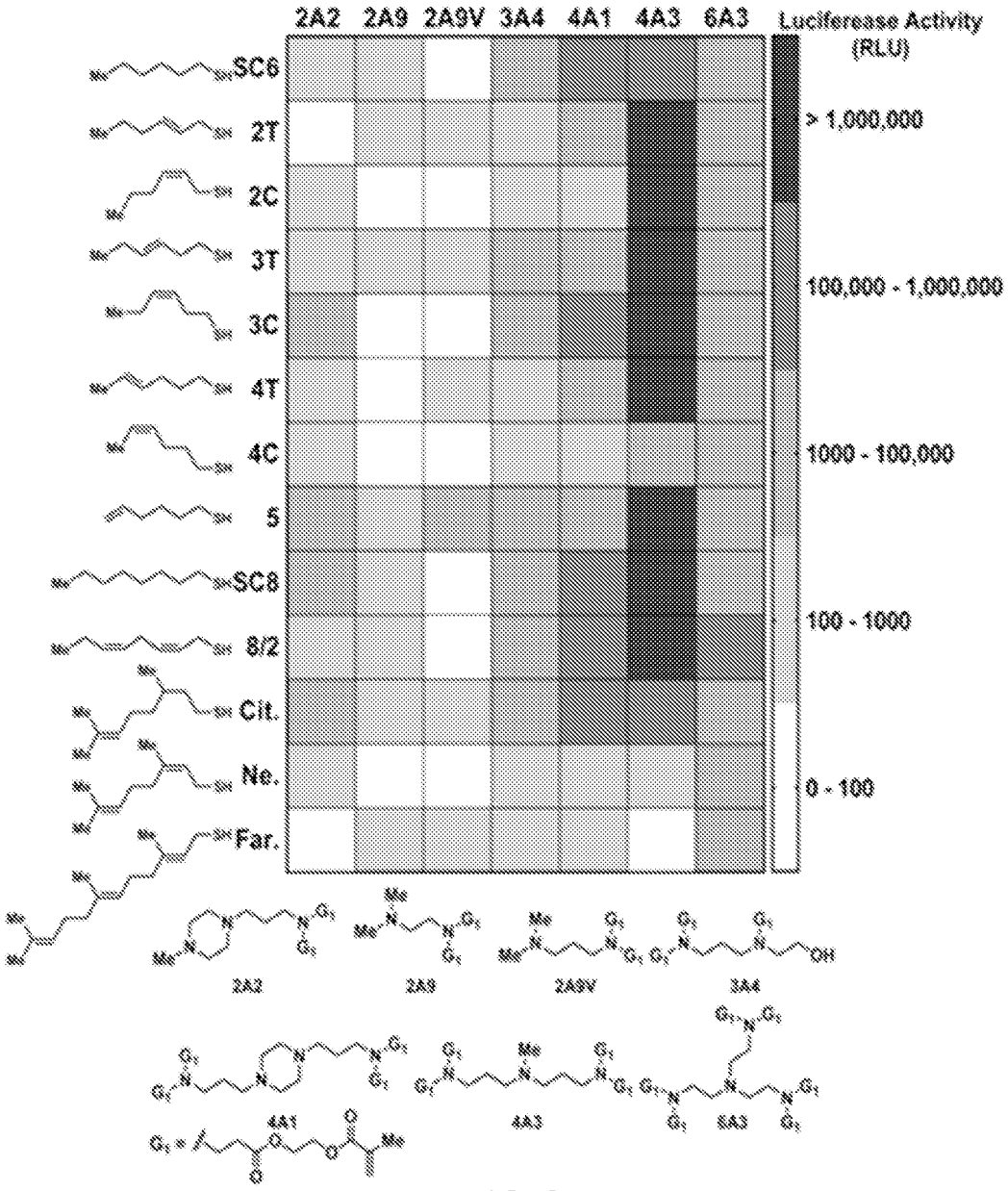

2A2
2A9
2A9V
3A4
4A1
4A3
6A3
Luciferease Activity (RLU)
SC6
2T
2C
3T
3C
4T
4C
5
SC8
8/2
Clt.
Ne.
Far.
> 1,000,000
100,000 - 1,000,000
1000 - 100,000
100 - 1000
0 - 100

FIG. 2B

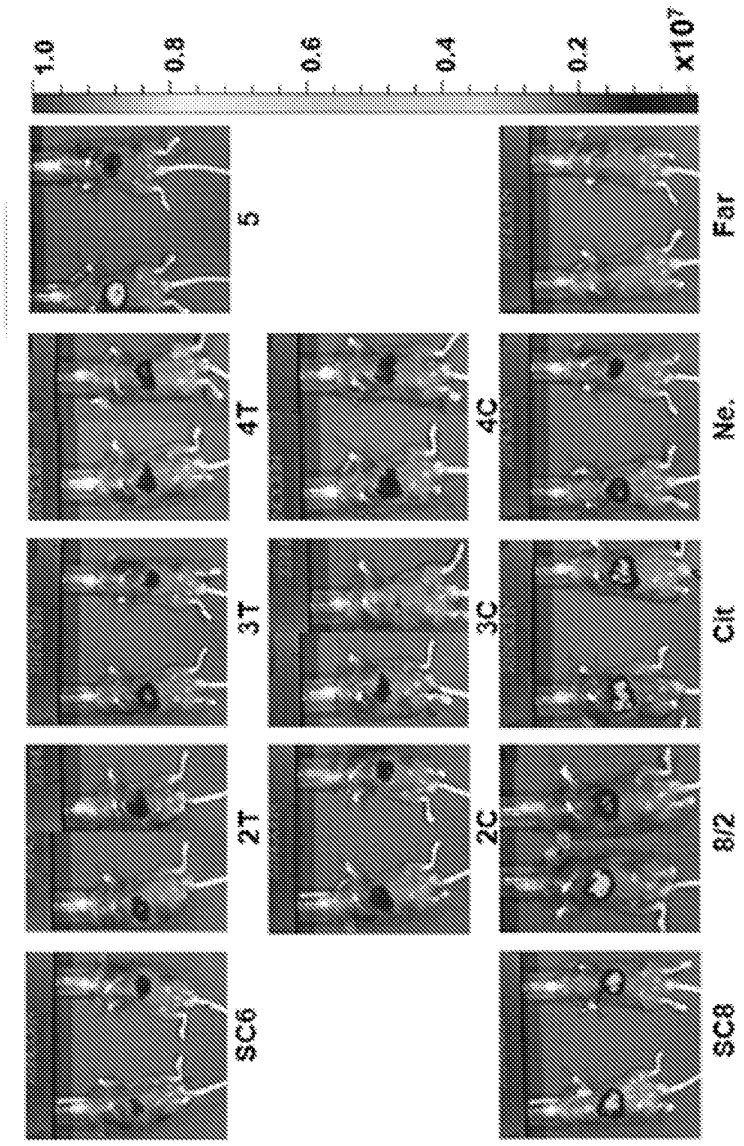


FIG. 3A

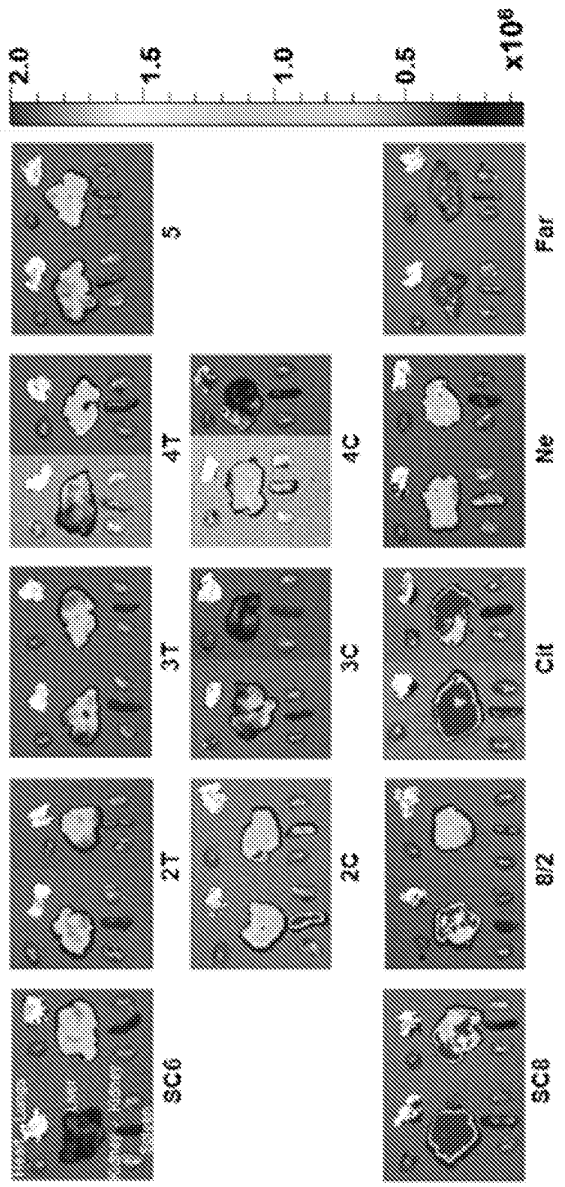


FIG. 3B

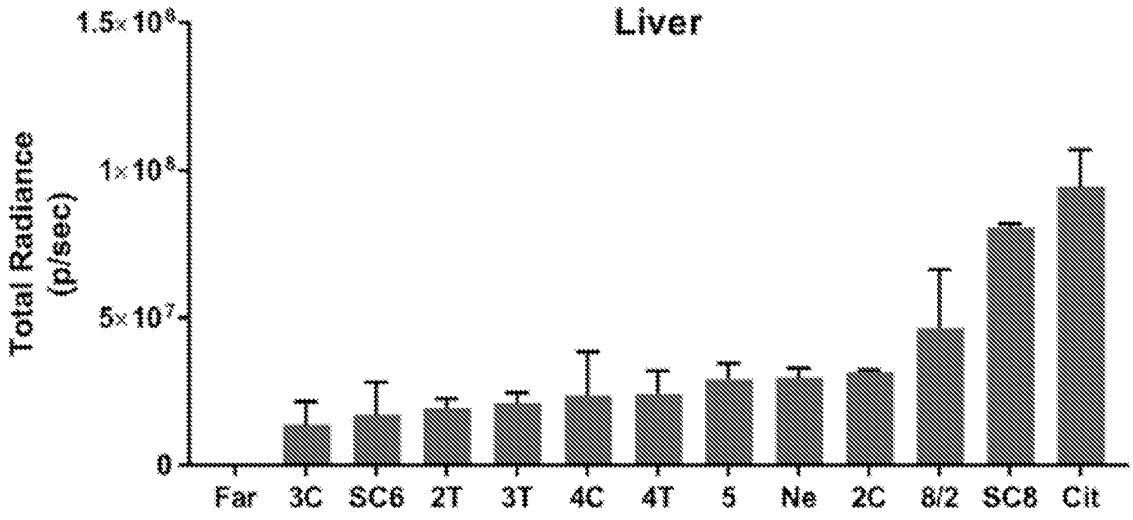

Liver
Total Radiance (p/sec)
1.5×10^8
1×10^8
5×10^7
0
Far
3C
SC6
2T
3T
4C
4T
5
Ne
2C
8/2
SC8
Cit

FIG. 3C

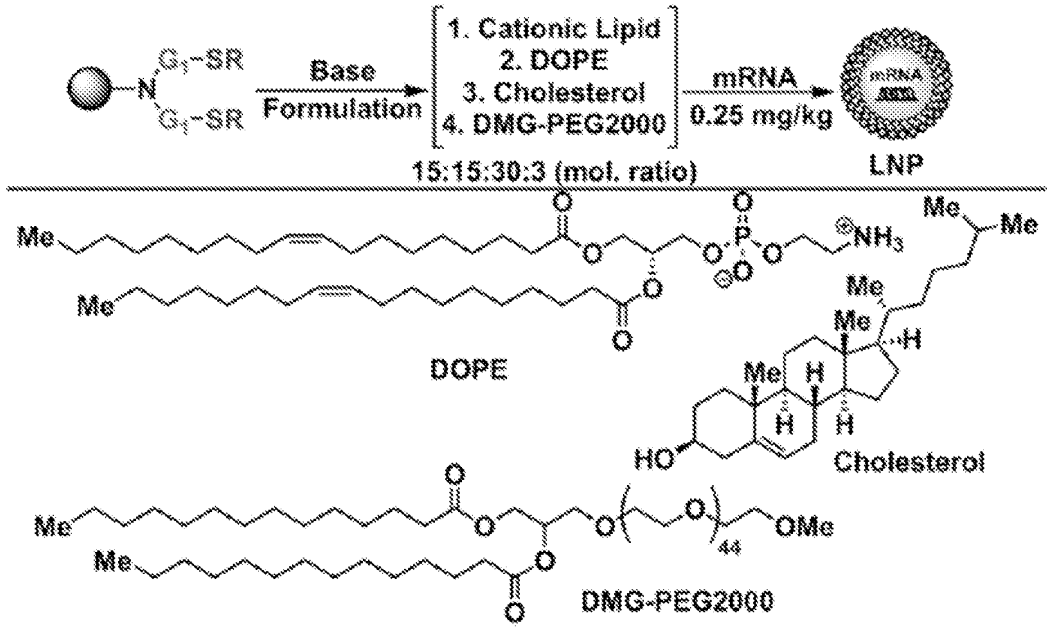

G1-SR
N
G1-SR
Base
Formulation
1. Cationic Lipid
2. DOPE
3. Cholesterol
4. DMG-PEG2000
15:15:30:3 (mol. ratio)
mRNA
0.25 mg/kg
LNP
DOPE
Cholesterol
DMG-PEG2000

FIG. 3D

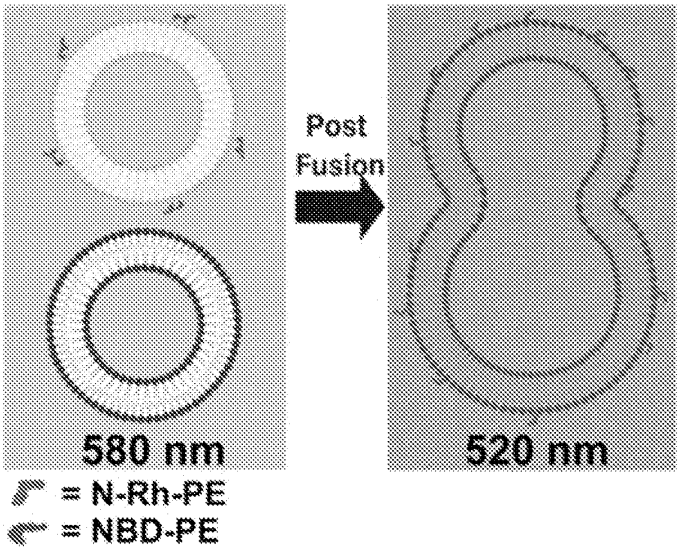

Post
Fusion
580 nm
520 nm
= N-Rh-PE
= NBD-PE

FIG. 4A

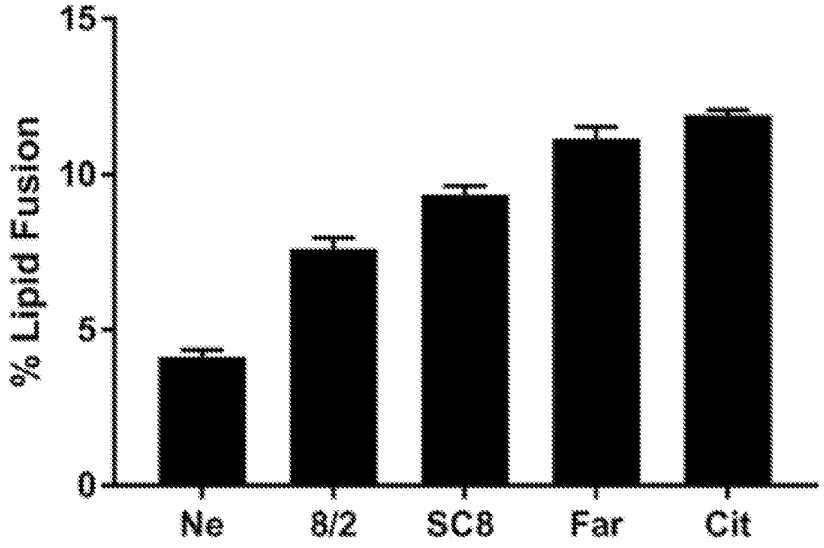

% Lipid Fusion
15
10
5
0
Ne
8/2
SC8
Far
Cit

FIG. 4B

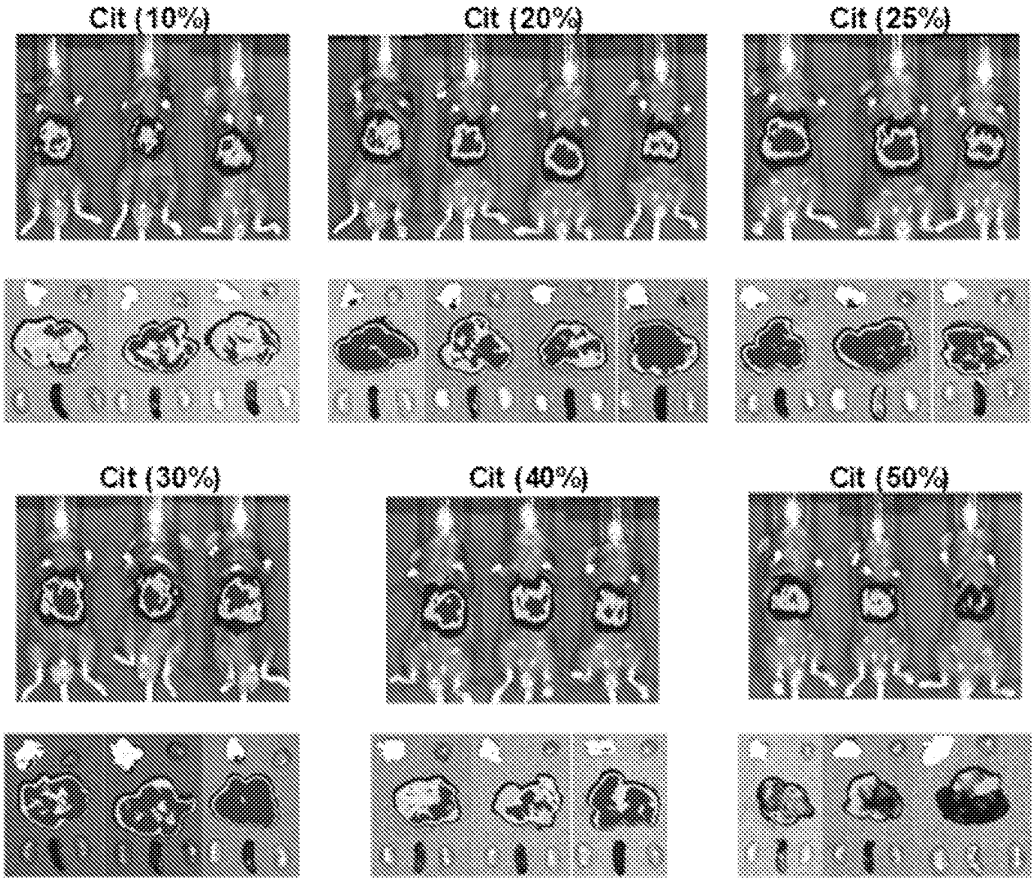


FIG. 5A

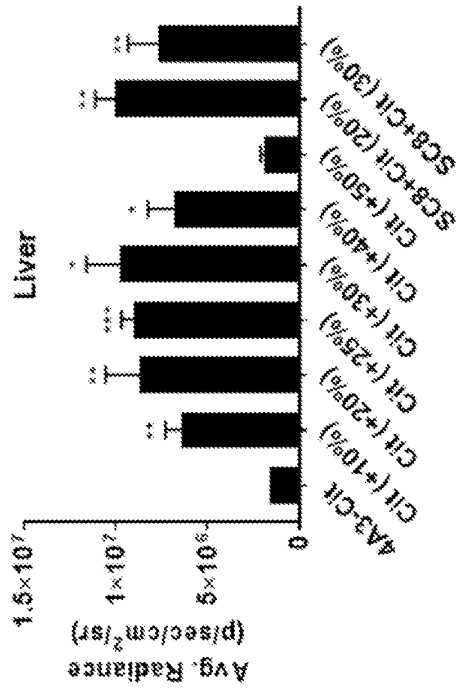

Liver
Avg. Radiance (p/sec/cm²/sr)
1.5×10⁷
1×10⁷
5×10⁶
0
4A3-Cit
Cit (+10%)
Cit (+20%)
Cit (+25%)
Cit (+30%)
Cit (+40%)
Cit (+50%)
SC8+Cit (20%)
SC8+Cit (30%)

FIG. 5C

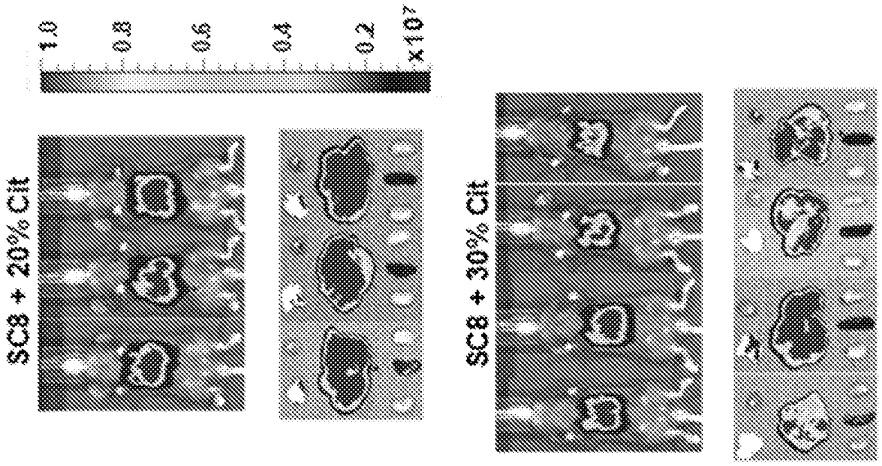

1.0
0.8
0.6
0.4
0.2
×10⁷
SC8 + 20% Cit
SC8 + 30% Cit

FIG. 5B

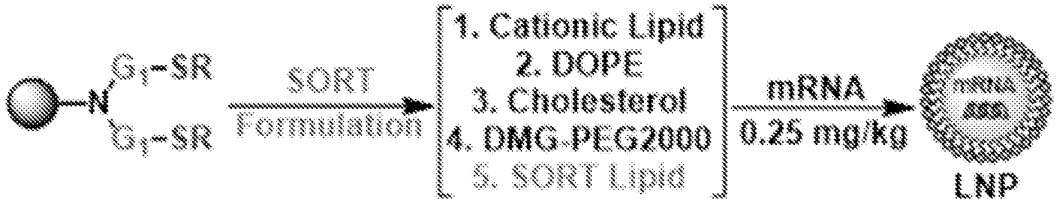


FIG. 5D

E	Molar Ratio (Molar %)					
	4A3-Cit	4A3-SC8	Added SORT Lipid 4A3-Cit	DOPE	Chol.	DMG-PEG2k
4A3-Cit (0%, Base)	15 (23.81)	0 (0)	0 (0)	15 (23.81)	30 (47.62)	3 (4.76)
Cit (+10%)	15 (21.43)	0 (0)	7 (10)	15 (21.43)	30 (42.86)	3 (4.29)
Cit (+20%)	15 (19.05)	0 (0)	15.75 (20)	15 (19.05)	30 (38.10)	3 (3.81)
Cit (+25%)	15 (17.86)	0 (0)	21 (25)	15 (17.86)	30 (35.71)	3 (3.57)
Cit (+30%)	15 (16.67)	0 (0)	27 (30)	15 (16.67)	30 (33.33)	3 (3.33)
Cit (+40%)	15 (14.29)	0 (0)	42 (40)	15 (14.29)	30 (28.57)	3 (2.86)
Cit (+50%)	15 (11.9)	0 (0)	63 (50)	15 (11.9)	30 (23.81)	3 (2.38)
SC8 + Cit (20%)	0 (0)	15 (21.43)	15.75 (20)	15 (19.05)	30 (38.10)	3 (3.81)
SC8 + Cit (30%)	0 (0)	15 (16.67)	27 (30)	15 (16.67)	30 (33.33)	3 (3.33)

FIG. 5E

Molar Ratio (Molar %)					
	Parent	Added Lipid	DOPE	Chol.	DMG-PEG2k
0%, Base	15 (23.81)	0 (0)	15 (23.81)	30 (47.62)	3 (4.76)
+5%	15 (22.62)	3.315 (5)	15 (22.62)	30 (45.24)	3 (4.52)
+10%	15 (21.43)	7 (10)	15 (21.43)	30 (42.86)	3 (4.29)
+20%	15 (19.05)	15.75 (20)	15 (19.05)	30 (38.10)	3 (3.81)
+25%	15 (17.86)	21 (25)	15 (17.86)	30 (35.71)	3 (3.57)
+30%	15 (16.67)	27 (30)	15 (16.67)	30 (33.33)	3 (3.33)
+40%	15 (14.29)	42 (40)	15 (14.29)	30 (28.57)	3 (2.86)
+50%	15 (11.9)	63 (50)	15 (11.9)	30 (23.81)	3 (2.38)

FIG. 6

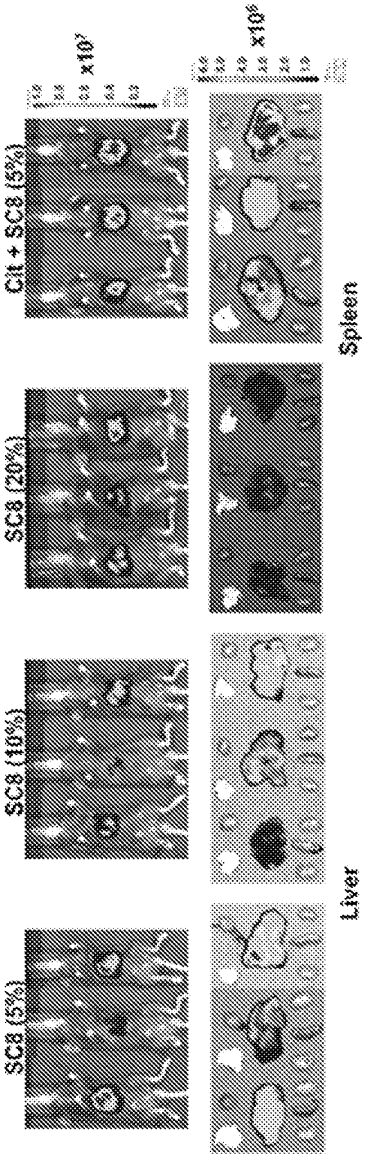

SC8 (5%)
SC8 (10%)
SC8 (20%)
Cit + SC8 (5%)
Liver
Spleen

FIG. 7A

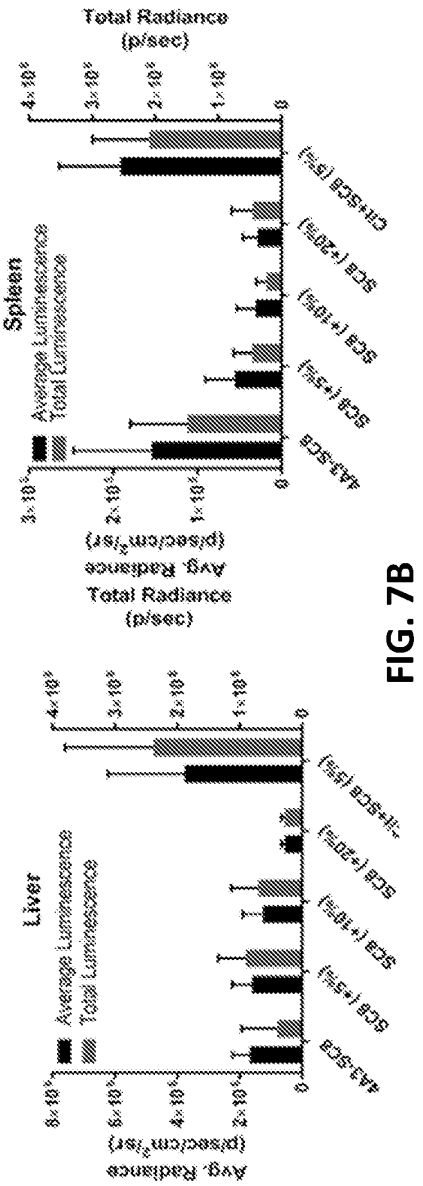

Total Radiance (p/sec)
Spleen
Average Luminescence
Total Luminescence
Avg. Radiance (p/sec/cm²/sr)
Liver
4A3-SCE
SCE (+5%)
SCE (+10%)
SCE (+20%)
Cit+SCE (5%)

FIG. 7B

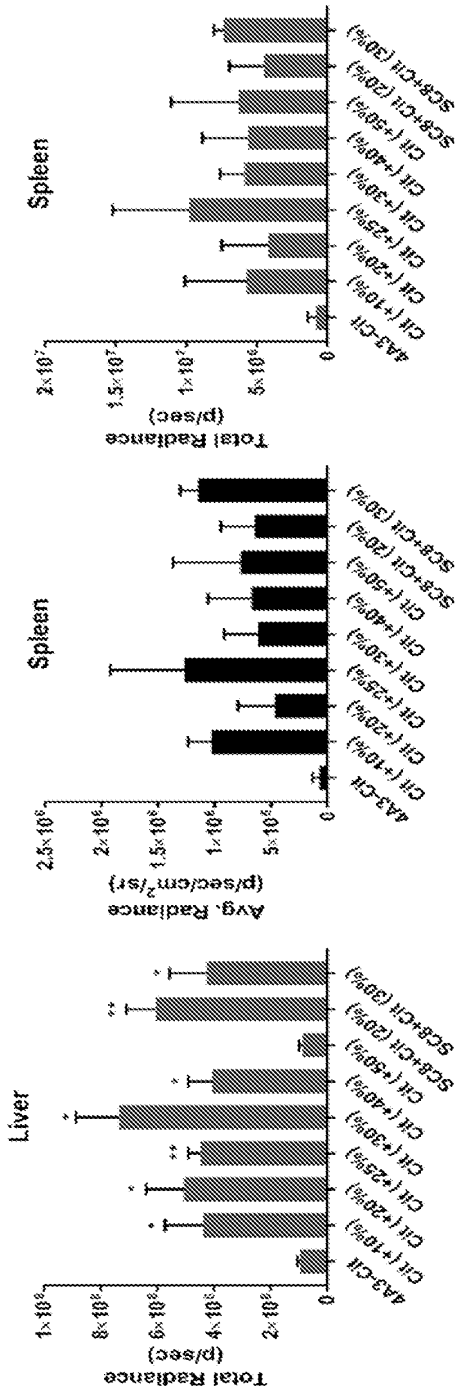

Liver
Total Radiance (p/sec)
Spleen
Avg. Radiance (p/sec/cm²/sr)
Spleen
Total Radiance (p/sec)
4A3-Cit
Cit (+10%)
Cit (+20%)
Cit (+25%)
Cit (+30%)
Cit (+40%)
Cit (+50%)
SCB+Cit (20%)
SCB+Cit (30%)

FIG. 8

	Diameter (nm)	PDI	E.E. (%)
4A3-SC6	121	0.193	97.2
4A3-2T	109	0.146	95.1
4A3-2C	97	0.190	96.7
4A3-3T	113	0.210	91.7
4A3-3C	98	0.188	94.1
4A3-4T	111	0.156	95.7
4A3-4C	130	0.105	95.2
4A3-5	99	0.151	93.2
4A3-SC8	146	0.153	93.3
4A3-8/2	123	0.160	94.5
4A3-Cit	143	0.186	90.0
4A3-Ne	131	0.159	96.3
4A3-Far	89	0.147	90.0

FIG. 9

	Diameter (nm)	PDI	E.E. (%)
4A3-SC8 (+5%)	146	0.182	97.7
SC8 (+10%)	139	0.147	95.5
SC8 (+20%)	163	0.202	96.6
Cit+SC8 (+5%)	144	0.191	96.7
Cit (+10%)	154	0.181	97.4
Cit (+20%)	163	0.164	98.1
Cit (+25%)	184	0.120	77.6
Cit (+30%)	172	0.163	96.9
Cit (+40%)	276	0.130	97.5
Cit (+50%)	276	0.125	96.5
SC8+Cit (+20%)	132	0.132	97.4
SC8+Cit (+30%)	234	0.125	96.0

FIG. 10

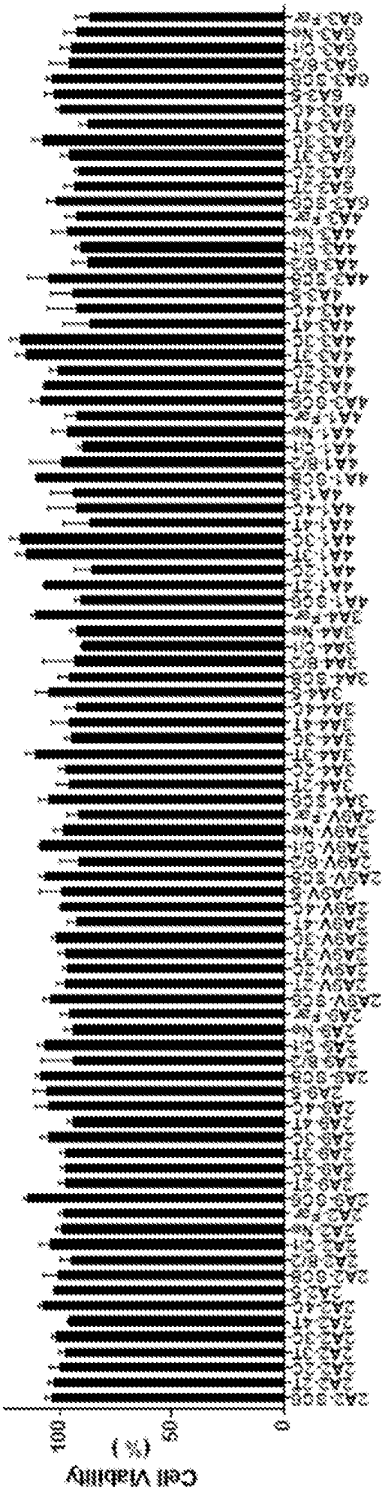


FIG. 11

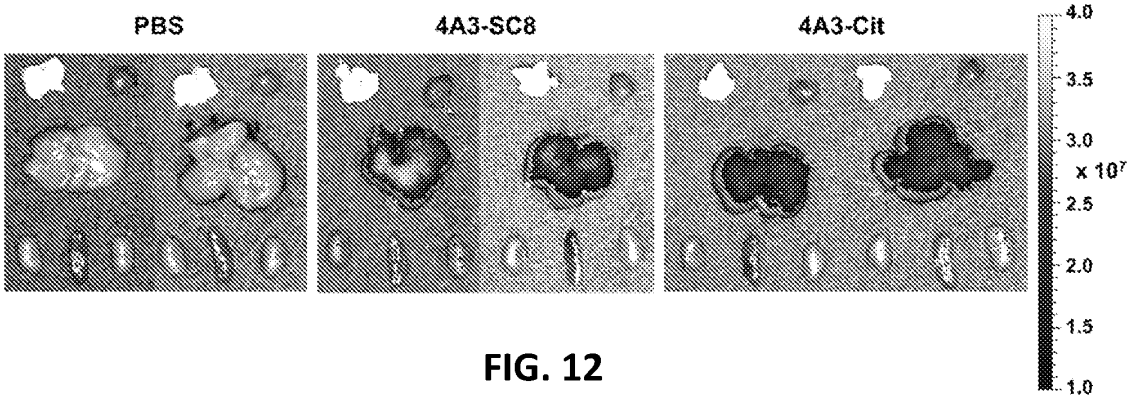

PBS
4A3-SC8
4A3-Cit
4.0
3.5
3.0
x 10^7
2.5
2.0
1.5
1.0

FIG. 12

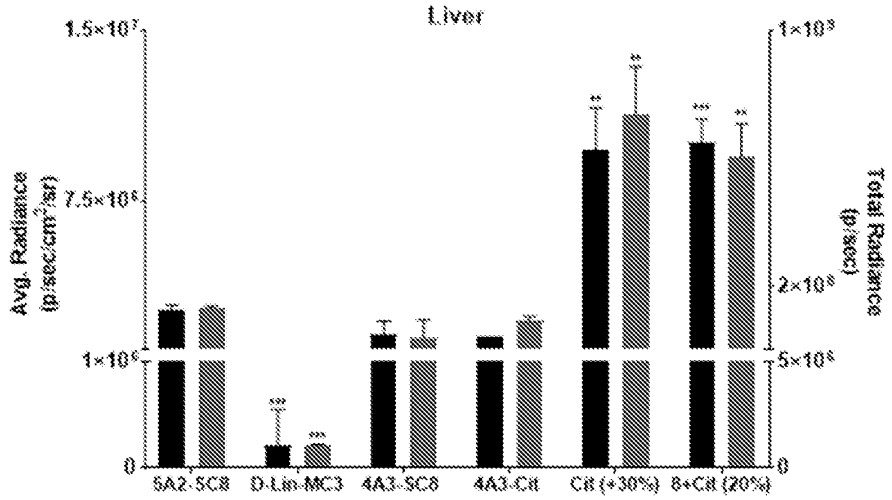

Liver
Avg. Radiance (p/sec/cm²/sr)
Total Radiance (p/sec)
1.5×10⁷
7.5×10⁶
1×10⁶
0
1×10⁹
2×10⁸
5×10⁸
0
5A2-SC8
D-Lin-MC3
4A3-SC8
4A3-Cit
Cit (+30%)
8+Cit (20%)

FIG. 13

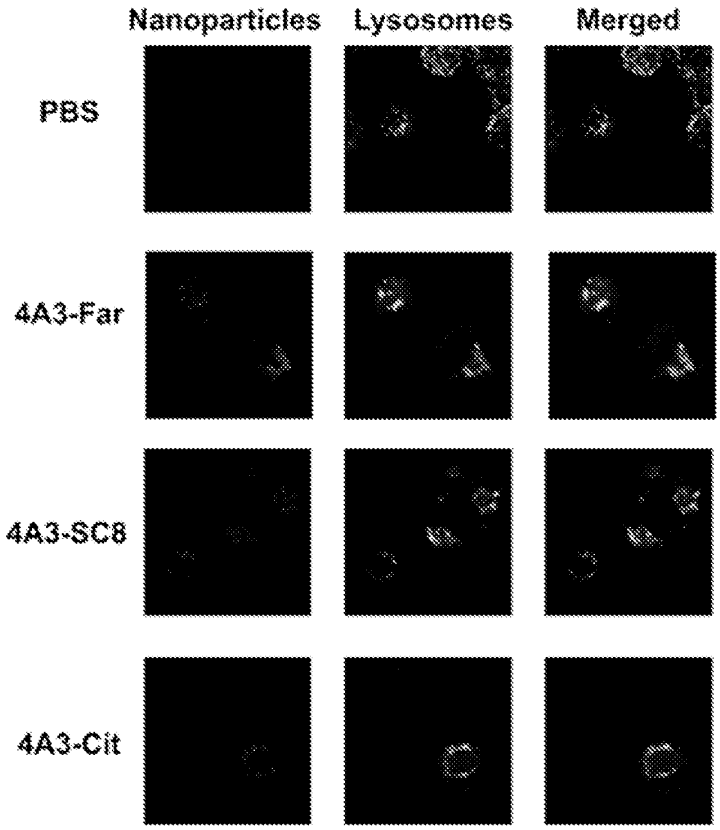


FIG. 14A

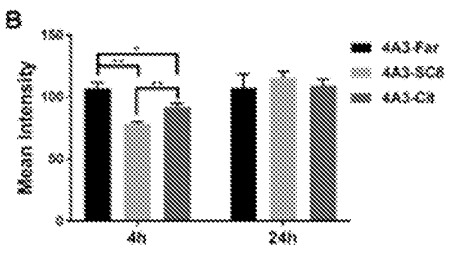


FIG. 14B

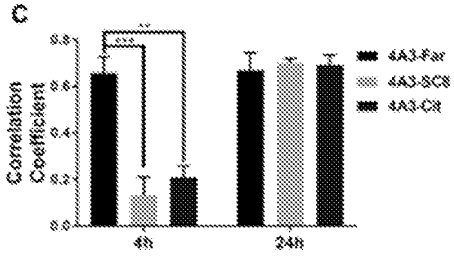


FIG. 14C