Supplementary Information (SI) for Nanoscale.

Liposome: a tool to raise the Cherenkov Radiation yield and to restore fluorophore properties in aqueous media

Supporting Information

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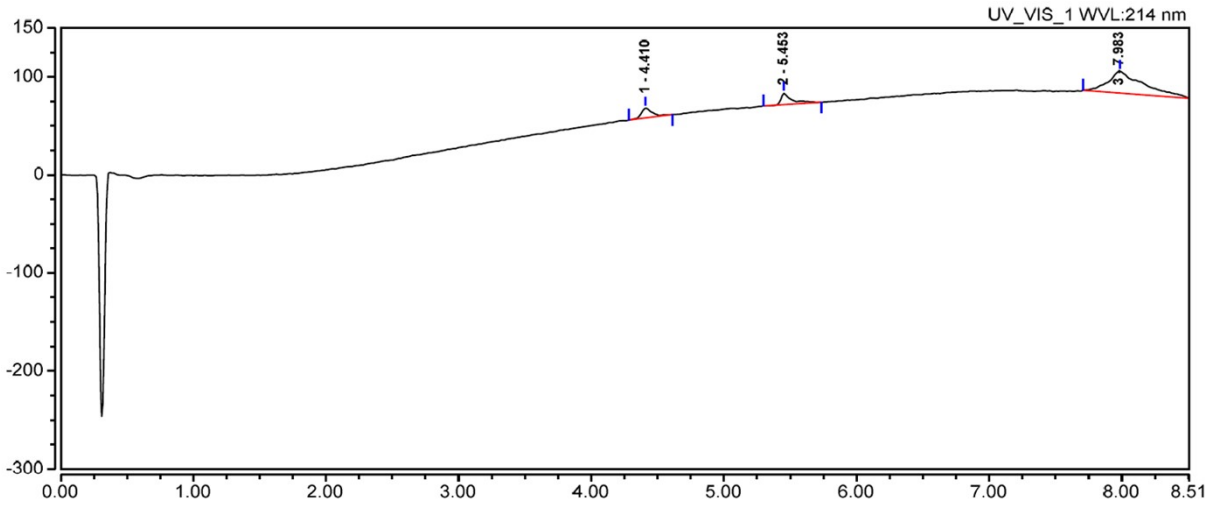
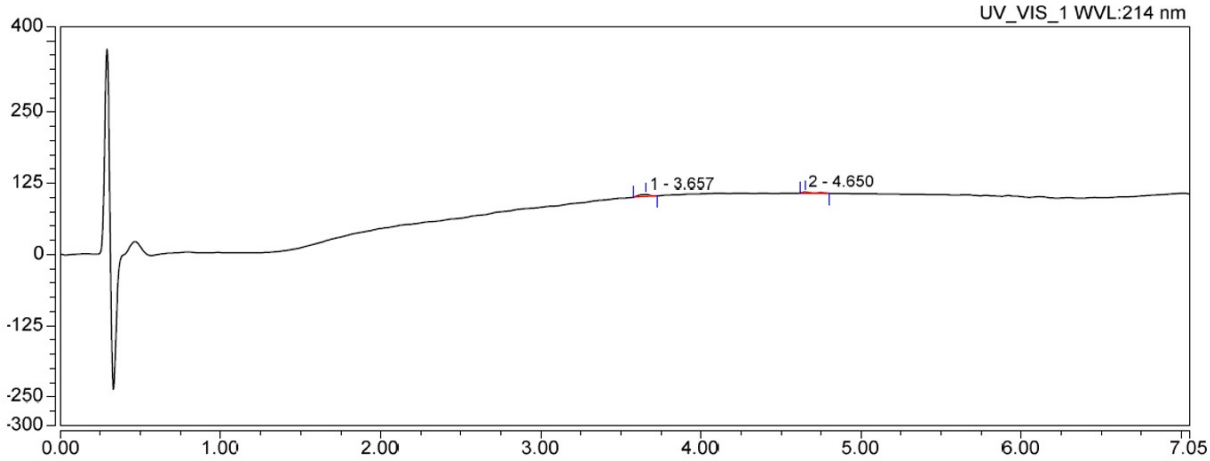
1. HPLC-MS column blank

HPLC-MS blank at 214 nm using the Phenomenex Kinetex C18 column.



HPLC-MS blank at 214 nm using the Phenomenex Kinetex C8 column.



2. Analytical data of synthesized compounds

Figure S1: ^{1}H NMR spectrum of NODAGA-NH-Tz-Cl (2) in D_2O (500 MHz) (* corresponds to DMF traces)

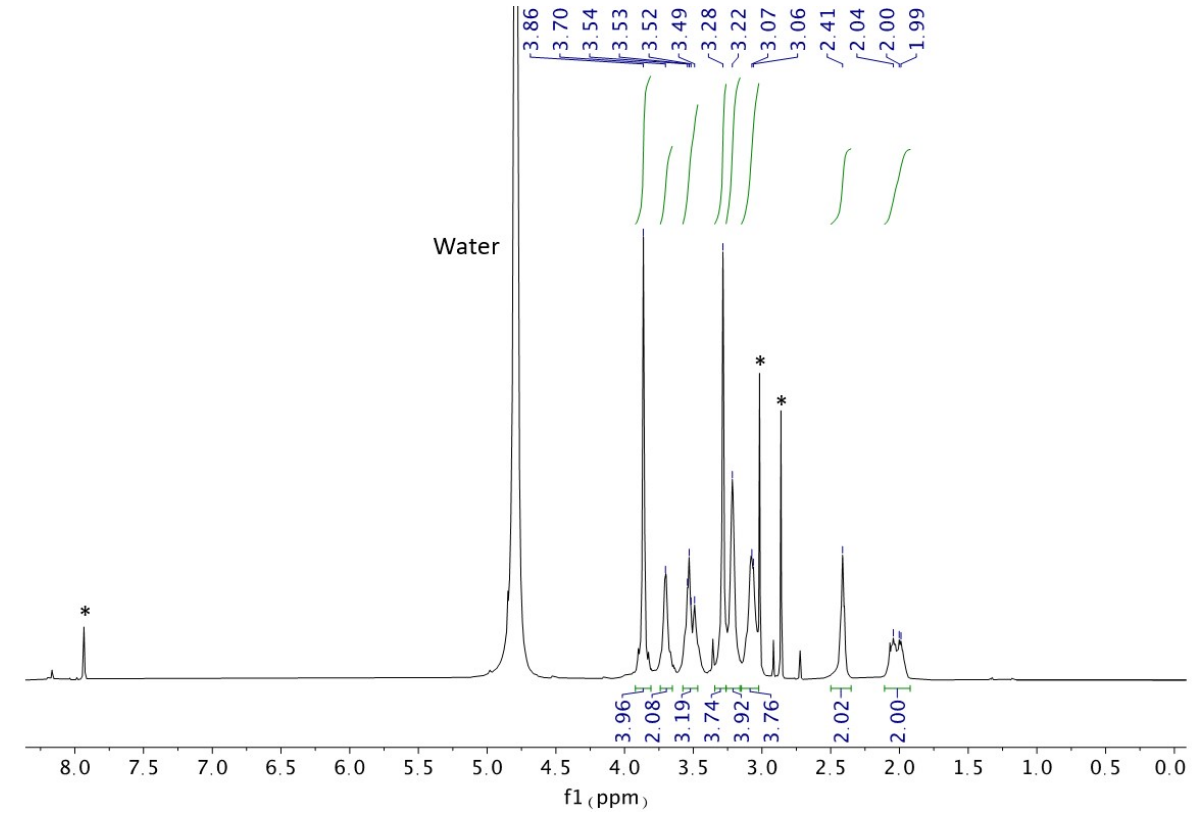


Figure S2: ^{13}C NMR spectrum of NODAGA-NH-Tz-Cl (2) in D_2O (126 MHz)

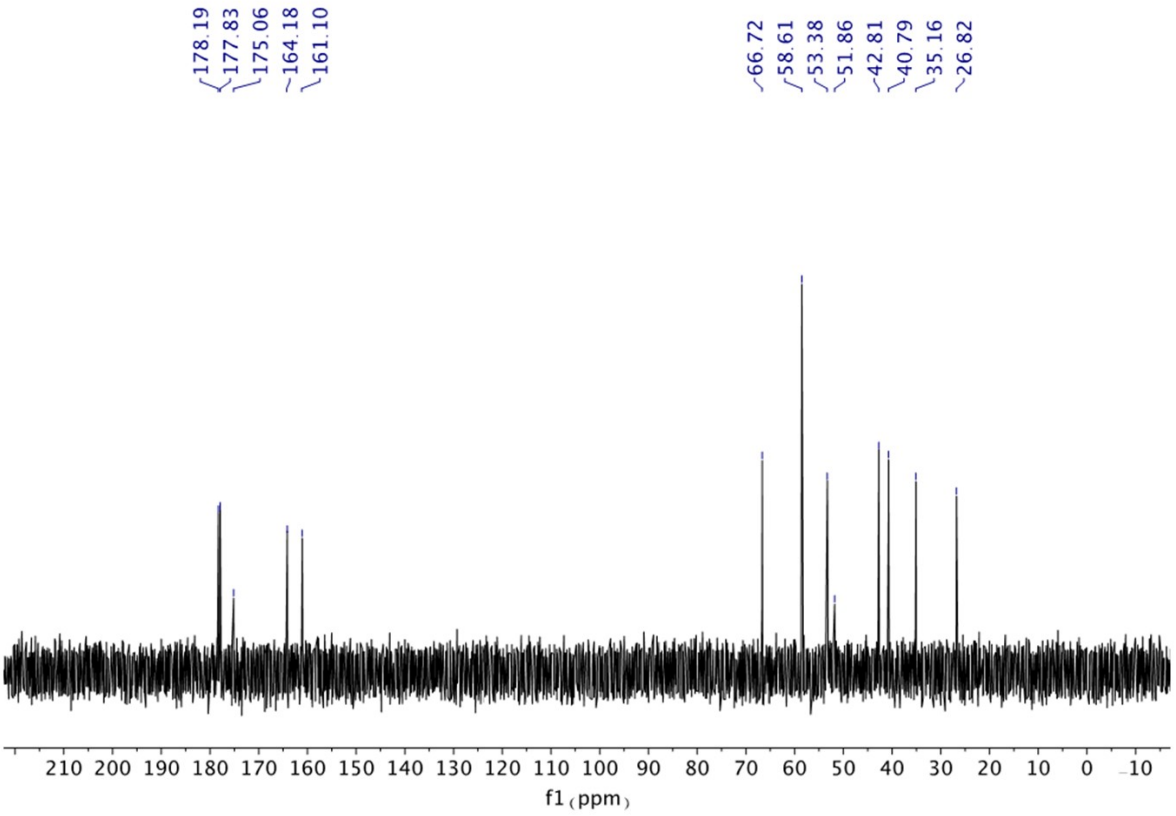


Figure S3: HPLC-MS chromatogram of NODAGA-NH-Tz-Cl (2) at 214 nm (system A, * corresponds to elution background)

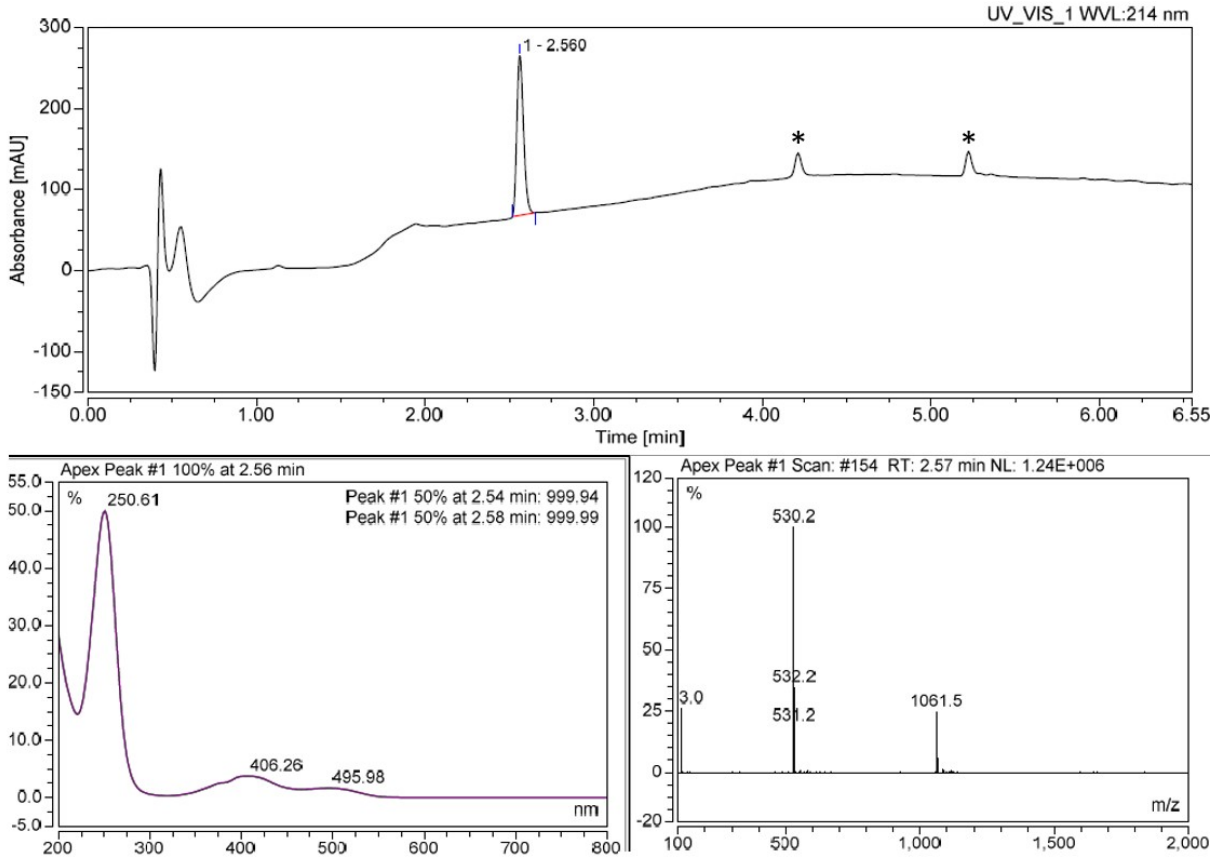


Figure S4: HPLC chromatogram of NODAGA-NH-Tz-Cl (2) at 400 nm (system A)

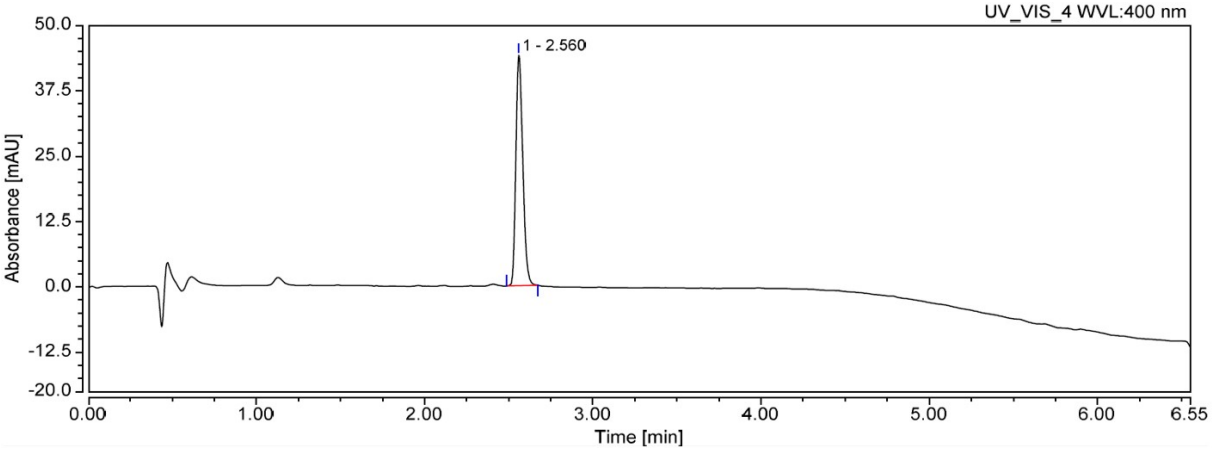


Figure S5: ^{1}H NMR spectrum of NODAGA-NH-Tz-cholesterol (1) in DMSO-*d6* (500 MHz)

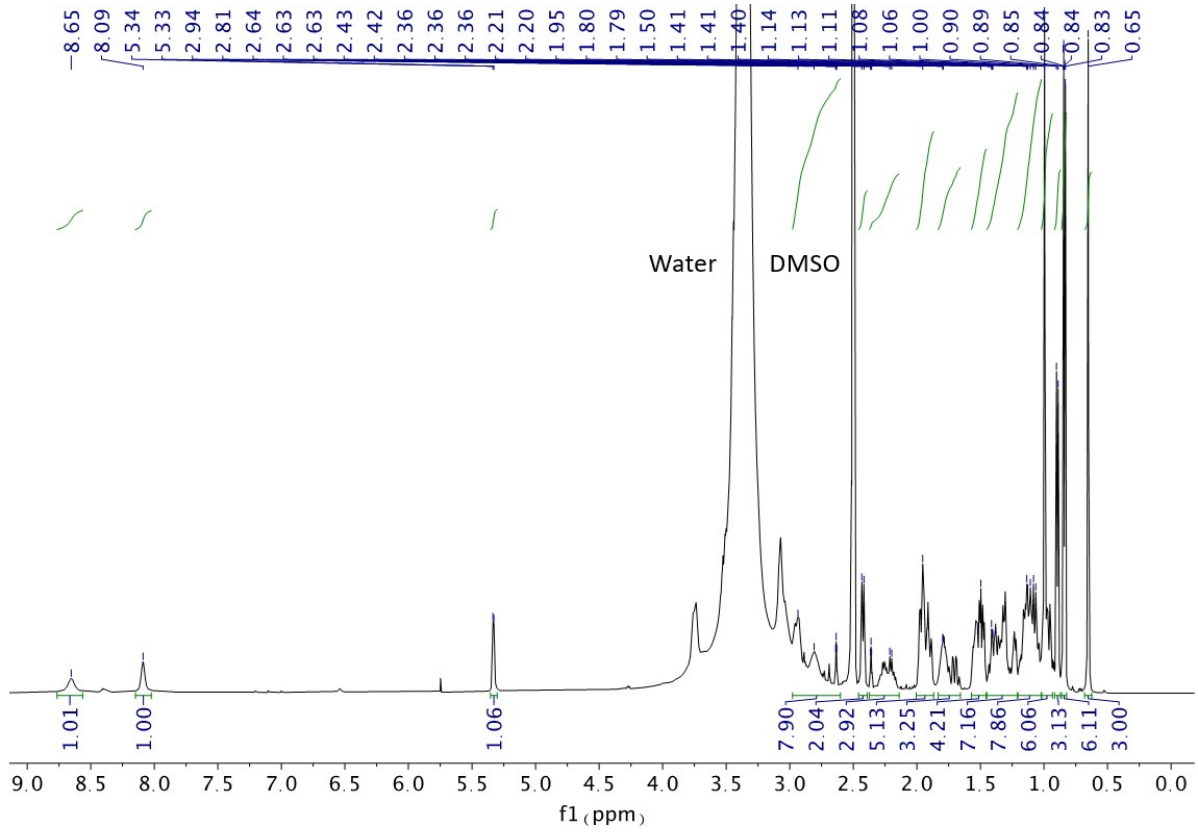


Figure S6: HPLC-MS chromatogram of NODAGA-NH-Tz-cholesterol (1) at 214 nm (system A, * corresponds to elution background)

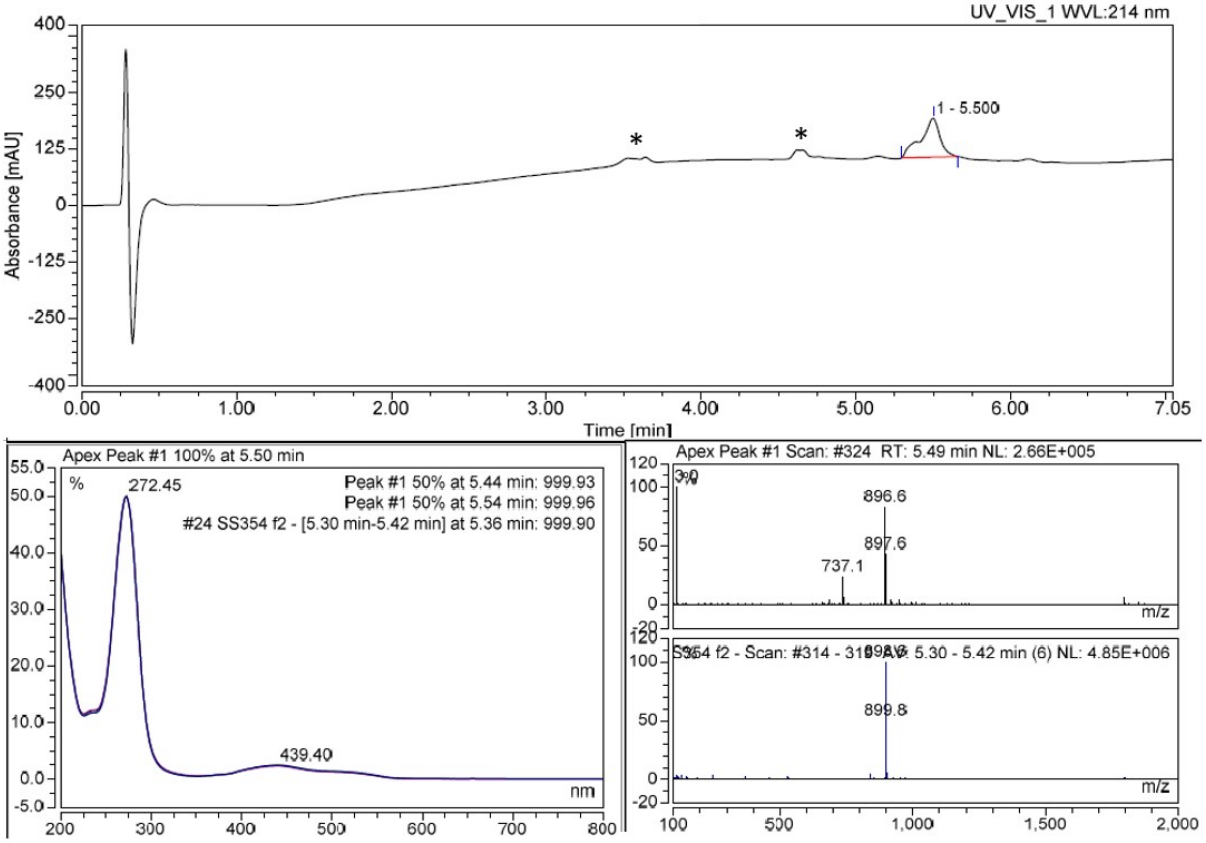


Figure S7: HPLC chromatogram of NODAGA-NH-Tz-cholesterol (1) at 400 nm

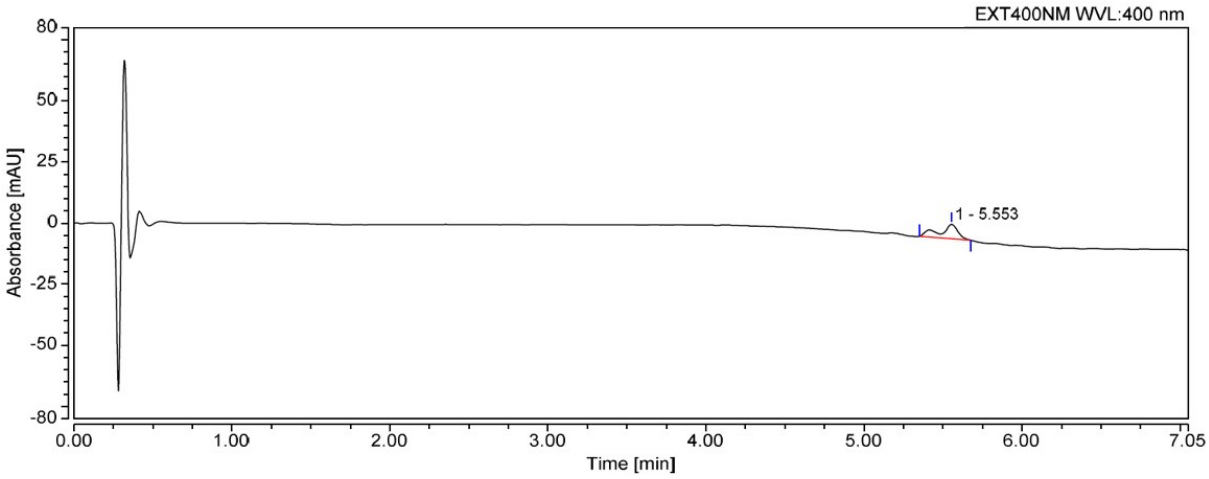
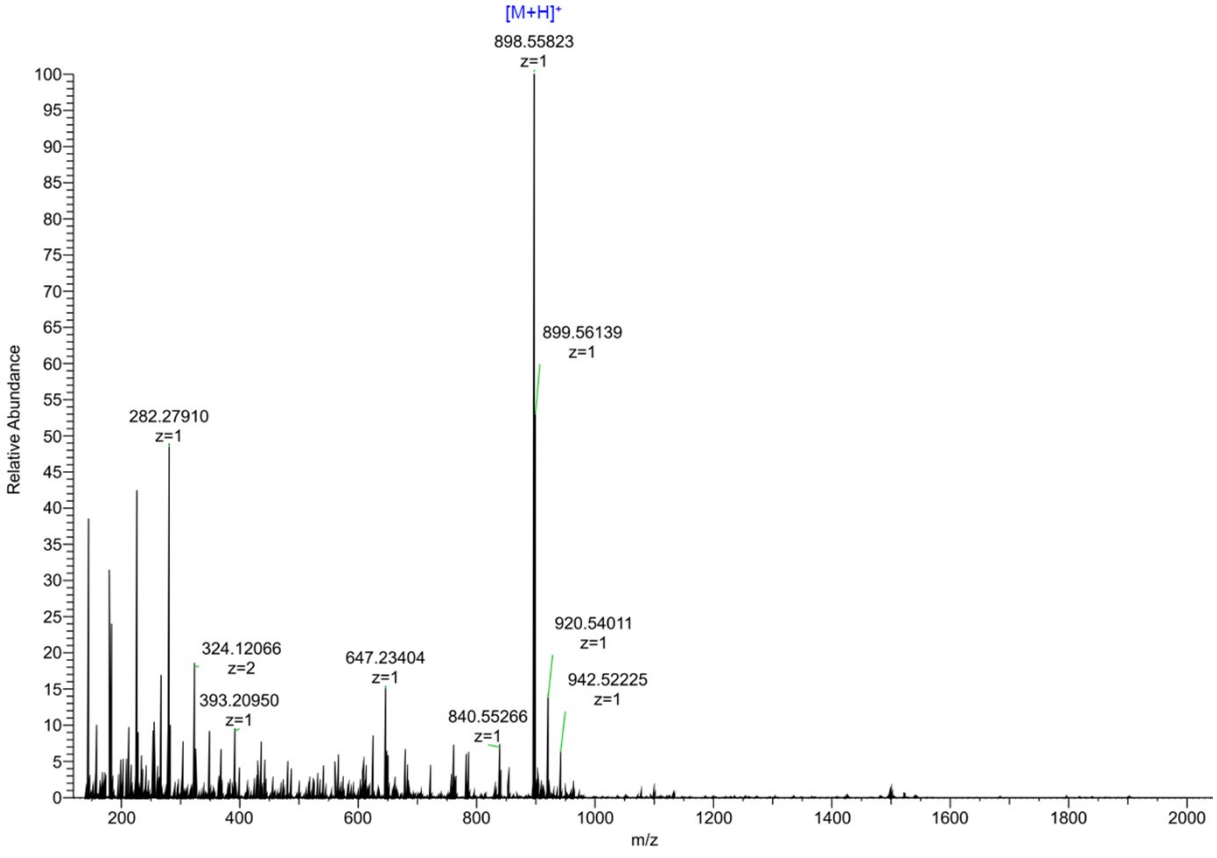
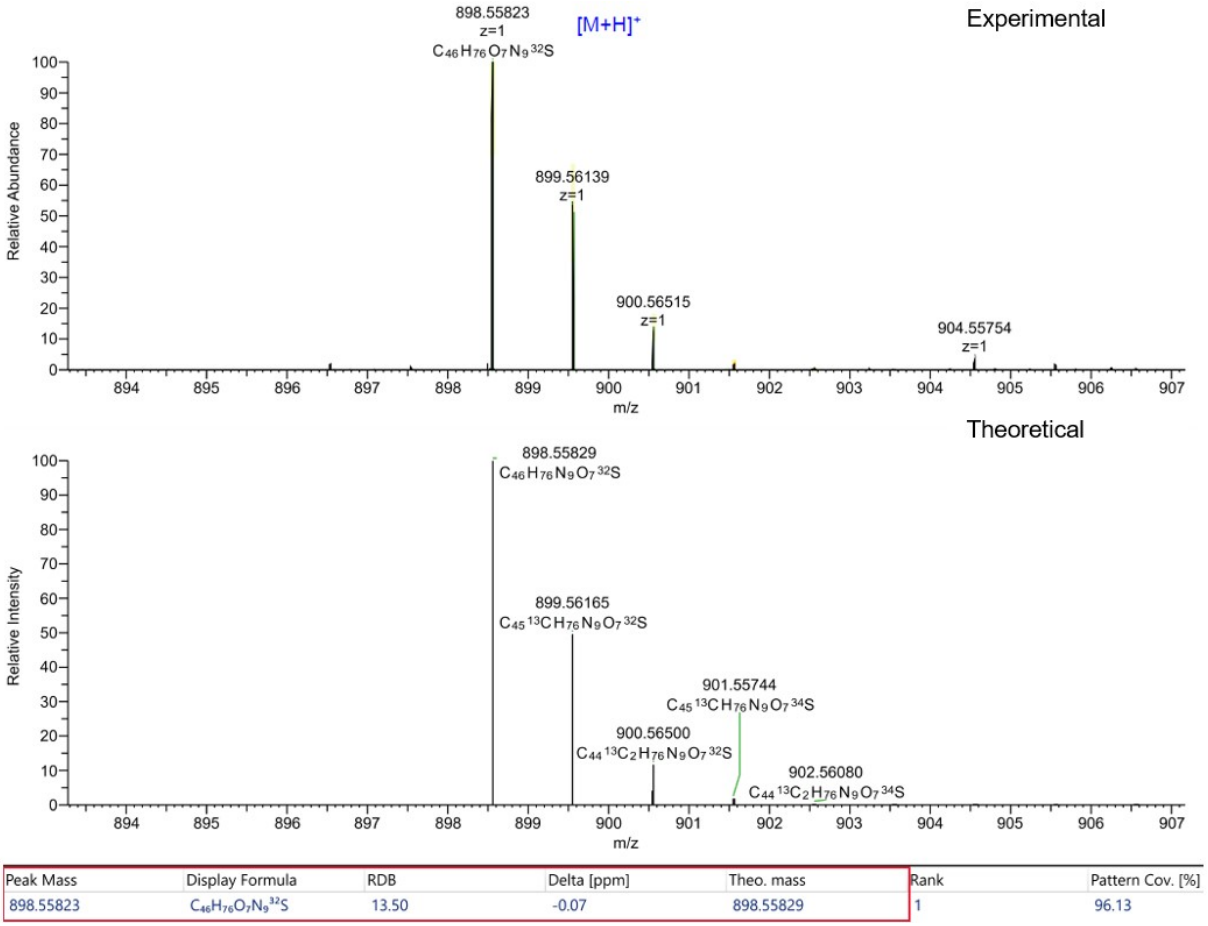


Figure S8: HRMS spectrum of NODAGA-NH-Tz-cholesterol (1)





Peak Mass	Display Formula	RDB	Delta [ppm]	Theo. mass	Rank	Pattern Cov. [%]
898.55823	$C_{46}H_{76}O_7N_9{}^{32}S$	13.50	-0.07	898.55829	1	96.13

Figure S9: ^{1}H NMR spectrum of 4-pyraninephthalonitrile (4) in MeOD (500 MHz)

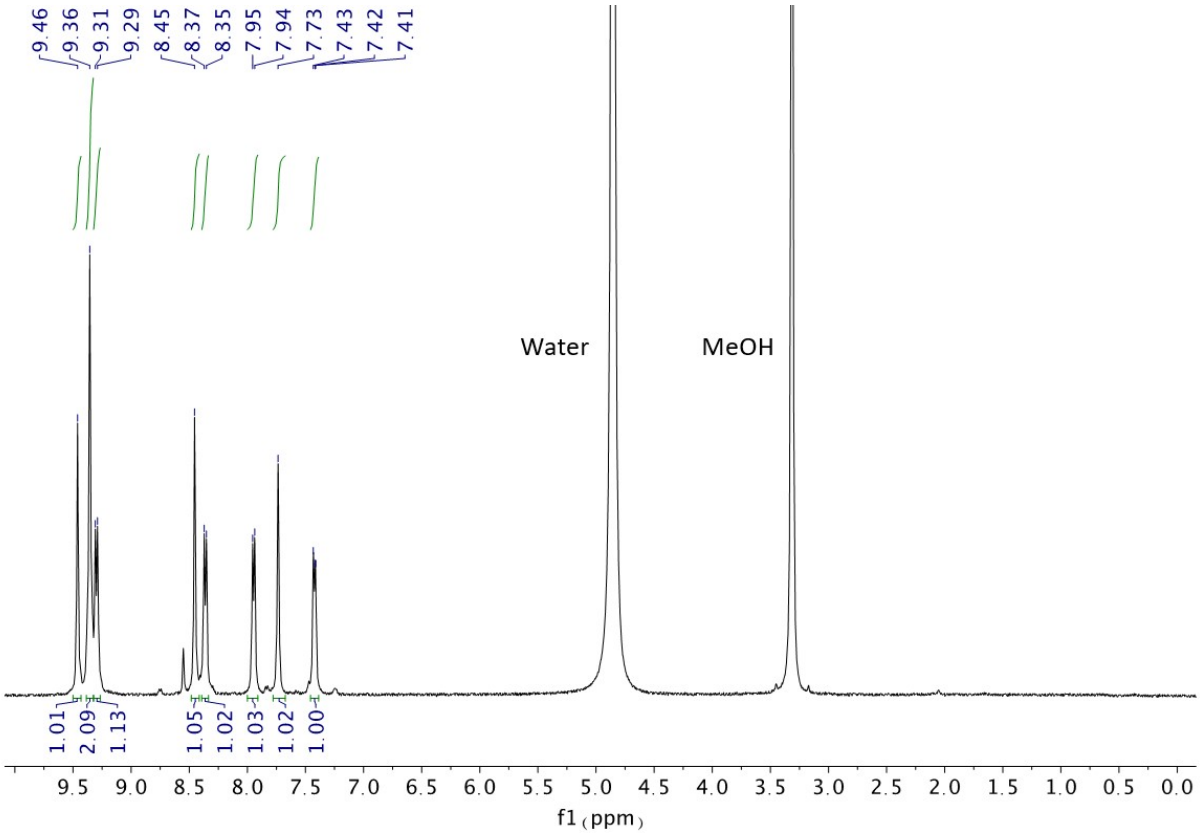


Figure S10: ^{13}C NMR spectrum of 4-pyraninephthalonitrile (4) in MeOD (126 MHz)

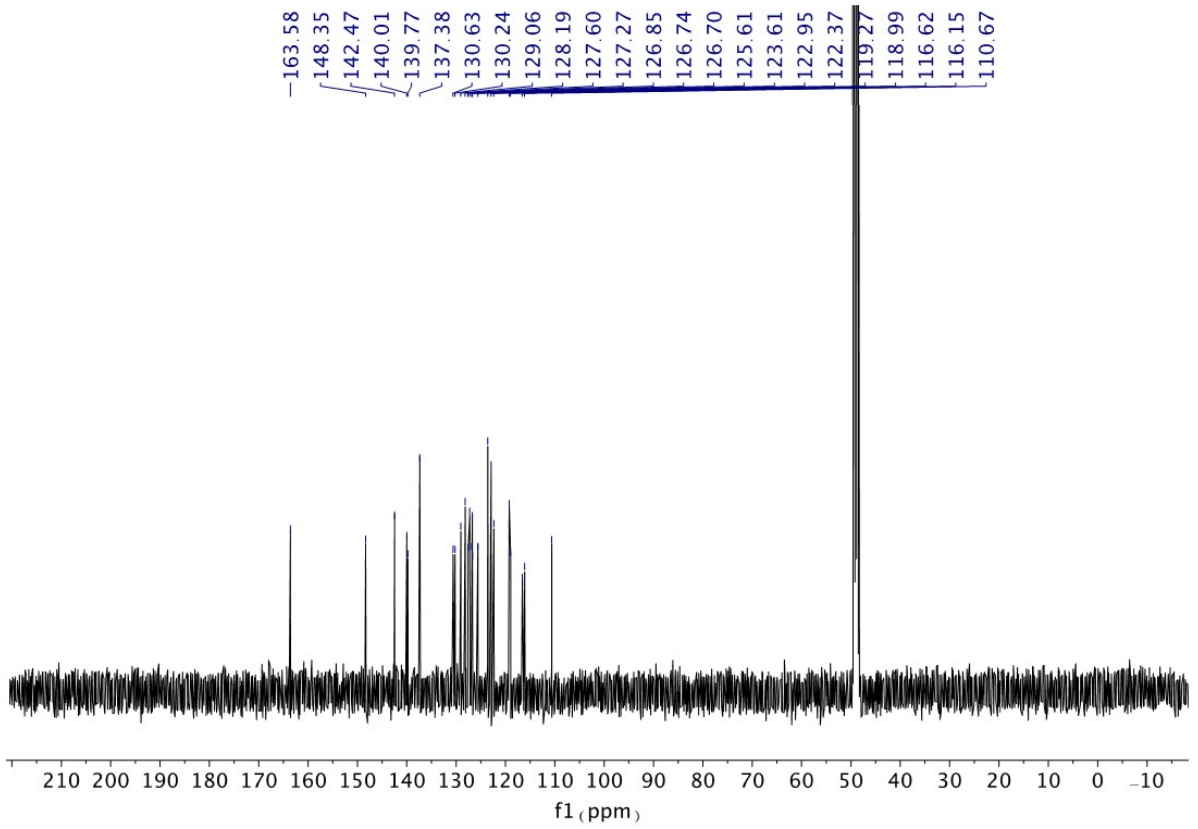
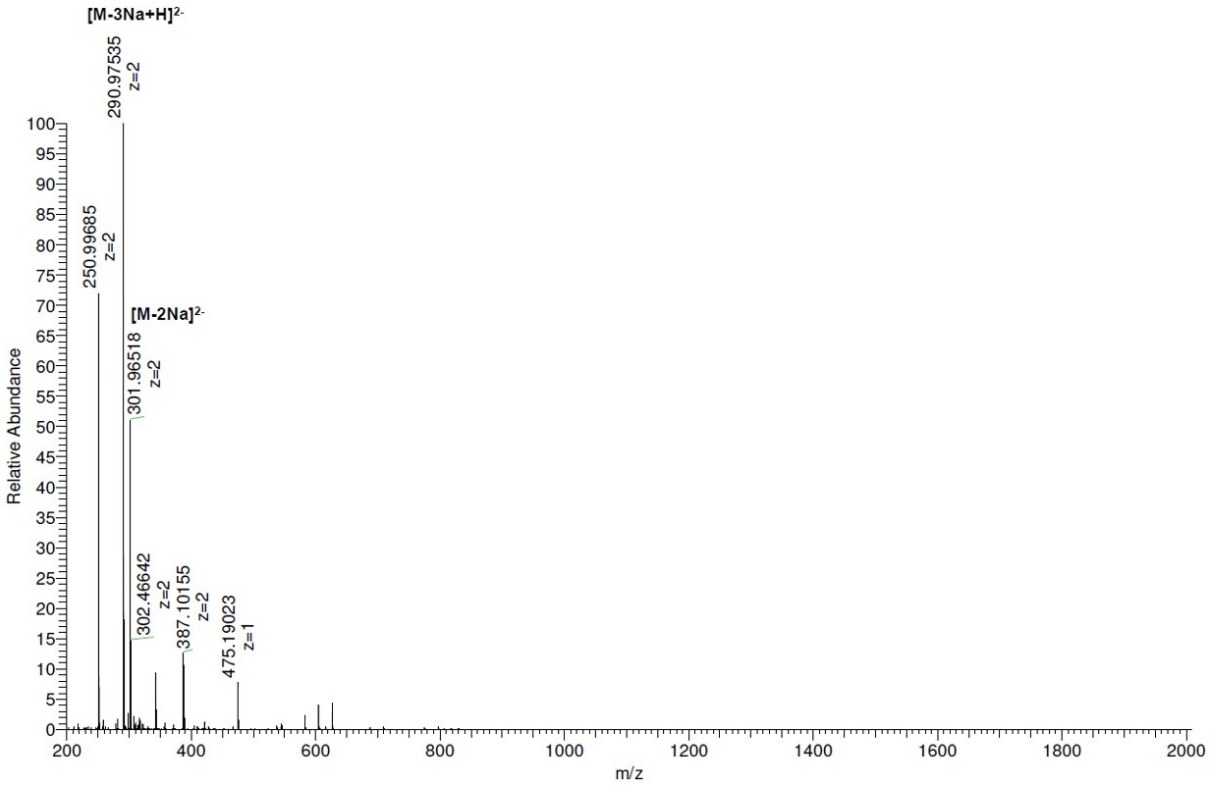


Figure S11: HRMS spectrum of 4-pyraninephthalonitrile (4)



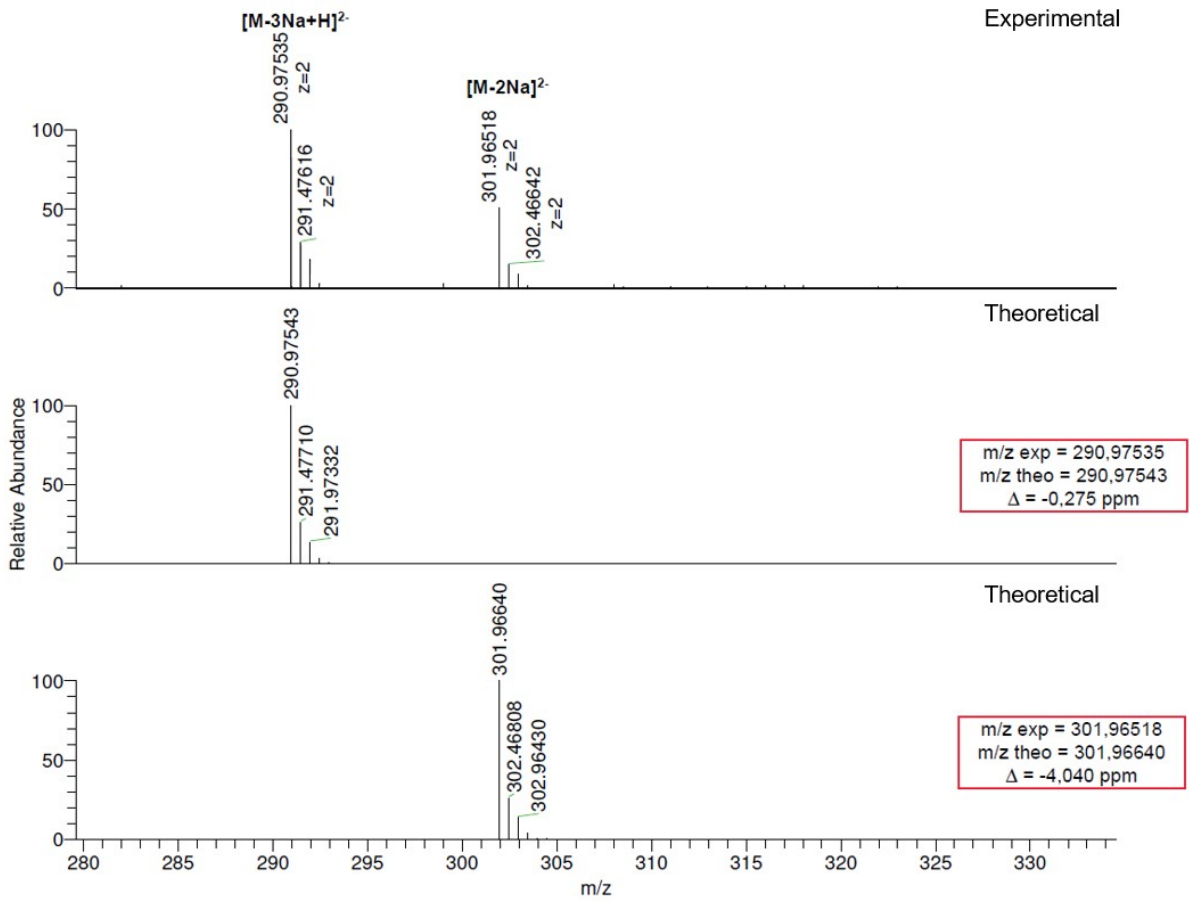


Figure S12: ^{1}H NMR spectrum of 4-pyraninephthalonitrile $(TBA)_3$ (5) in MeOD (500 MHz)

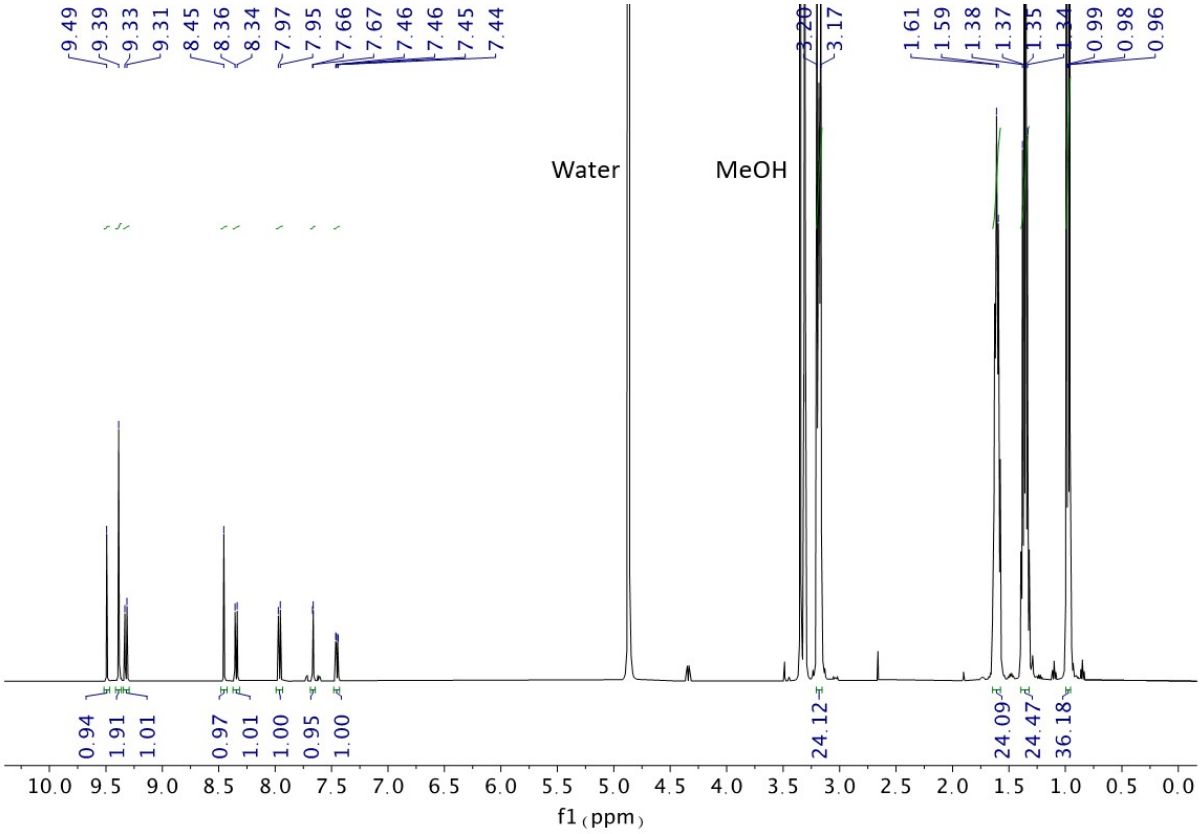


Figure S13: ^{1}H NMR spectrum of Pyranine-phthalocyanine (3) in MeOD (500 MHz)

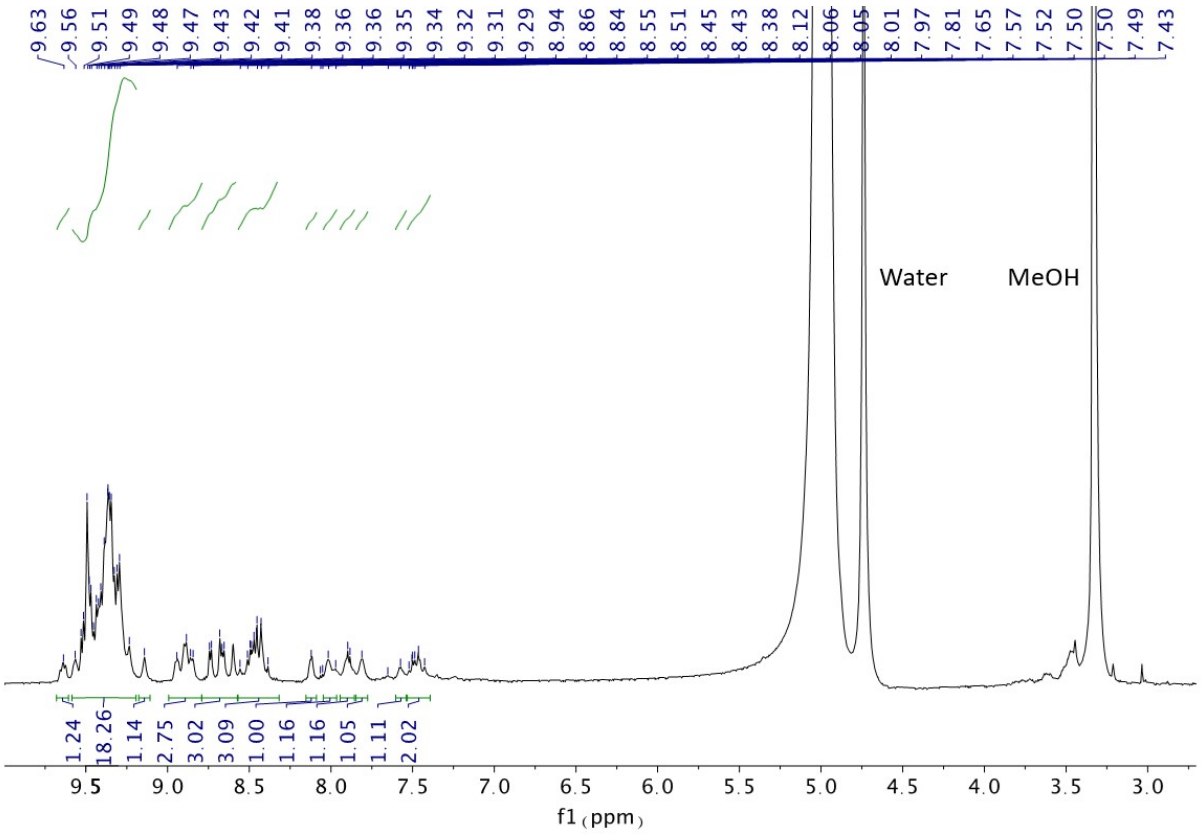


Figure S14: HPLC chromatogram of Pyranine-phthalocyanine (3) at 214 nm and UV-Vis absorption spectrum

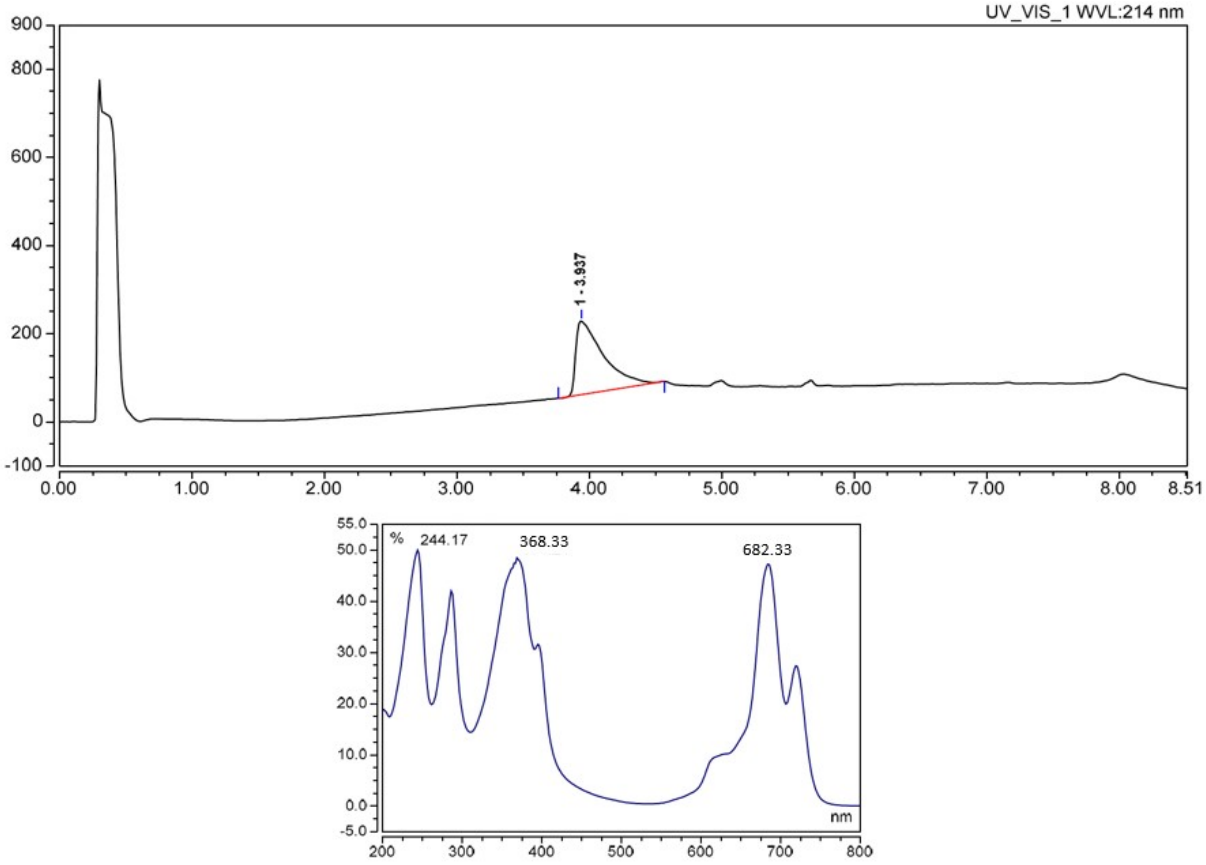


Figure S15: HPLC chromatogram of Pyranine-phthalocyanine (3) at 680 nm

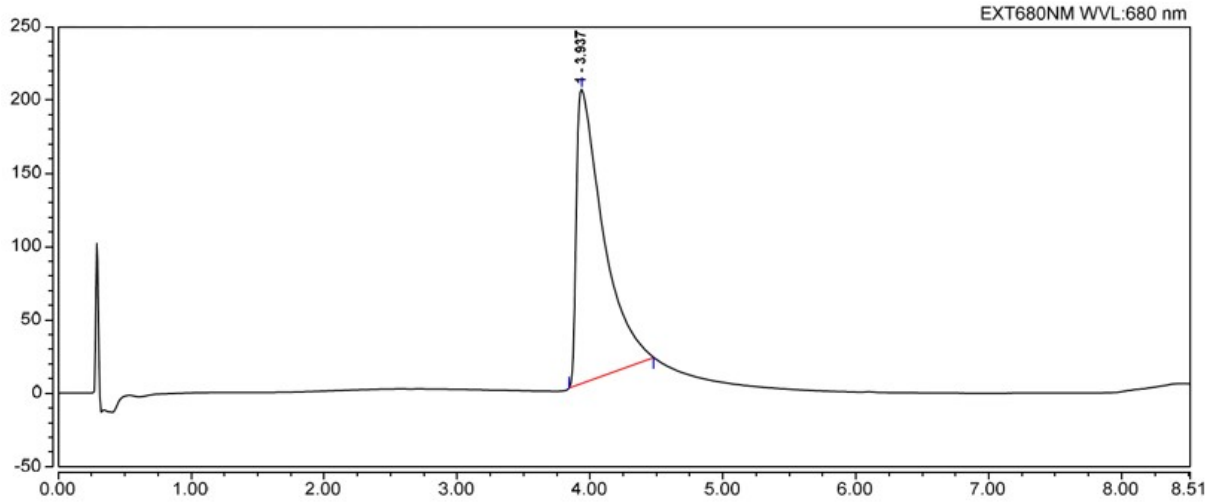
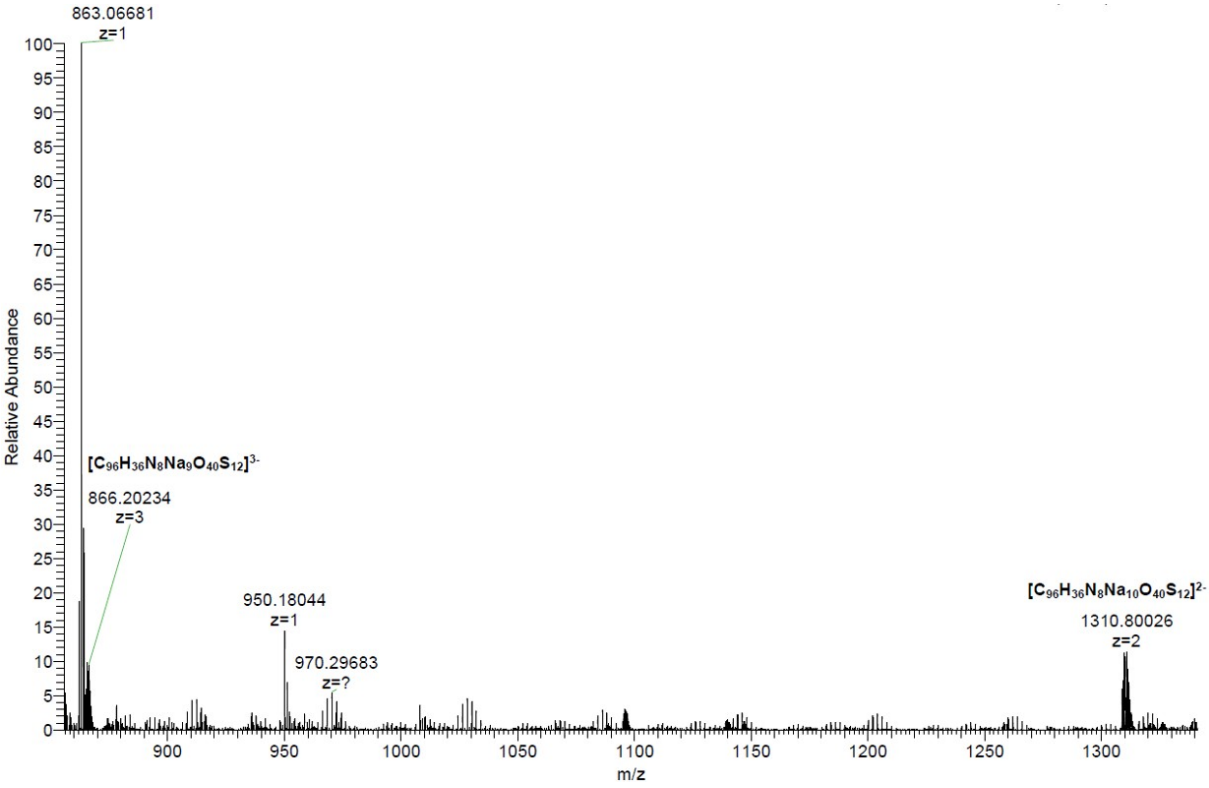
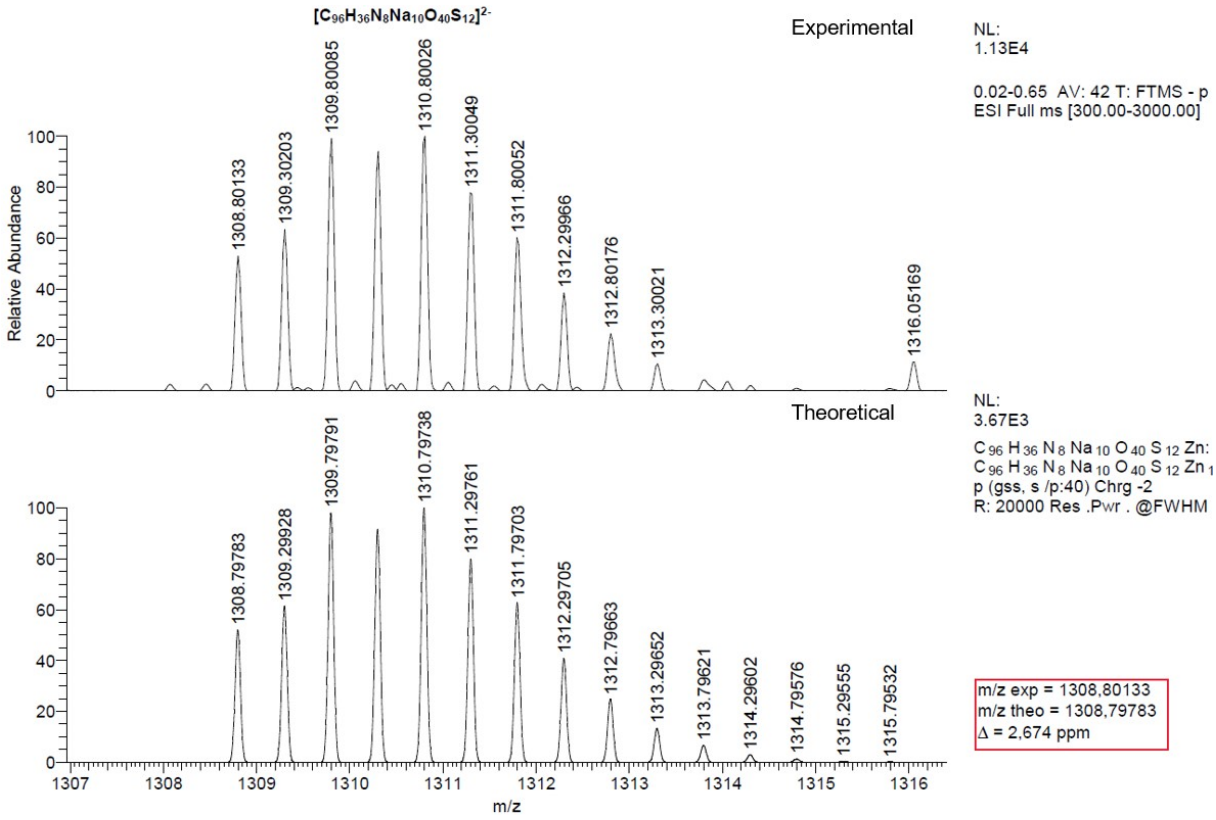
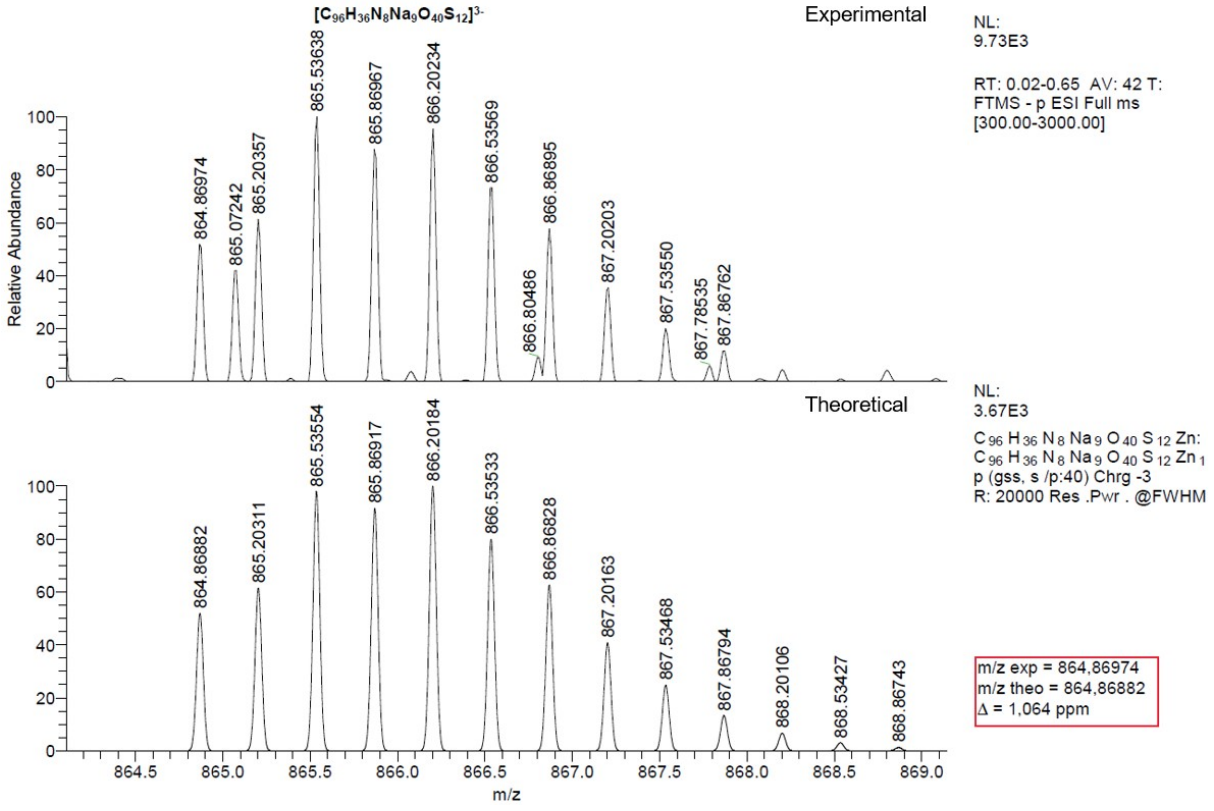


Figure S16: HRMS spectrum of Pyranine-phthalocyanine (3)




[C96H36N8Na10O40S12]2-
Experimental
NL:
1.13E4
0.02-0.65 AV: 42 T: FTMS - p
ESI Full ms [300.00-3000.00]
Relative Abundance
1308.80133
1309.30203
1309.80085
1310.80026
1311.30049
1311.80052
1312.29966
1312.80176
1313.30021
1316.05169
Theoretical
NL:
3.67E3
C96 H36 N8 Na10 O40 S12 Zn:
C96 H36 N8 Na10 O40 S12 Zn1
p (gss, s /p:40) Chrg -2
R: 20000 Res .Pwr . @FWHM
1308.79783
1309.29928
1309.79791
1310.79738
1311.29761
1311.79703
1312.29705
1312.79663
1313.29652
1313.79621
1314.29602
1314.79576
1315.29555
1315.79532
m/z exp = 1308,80133
m/z theo = 1308,79783
Δ = 2,674 ppm
m/z


[C96H36N8Na9O40S12]3-
Experimental
NL:
9.73E3
RT: 0.02-0.65 AV: 42 T:
FTMS - p ESI Full ms
[300.00-3000.00]
Relative Abundance
864.86974
865.07242
865.20357
865.53638
865.86967
866.20234
866.53569
866.80486
866.86895
867.20203
867.53550
867.78535
867.86762
Theoretical
NL:
3.67E3
C96 H36 N8 Na9 O40 S12 Zn:
C96 H36 N8 Na9 O40 S12 Zn1
p (gss, s /p:40) Chrg -3
R: 20000 Res .Pwr . @FWHM
864.86882
865.20311
865.53554
865.86917
866.20184
866.53533
866.86828
867.20163
867.53468
867.86794
868.20106
868.53427
868.86743
m/z exp = 864,86974
m/z theo = 864,86882
Δ = 1,064 ppm
m/z

3. Liposome characterisations

Figure S17: FPLC chromatogram of liposome encapsulating eosin Y (0 days)

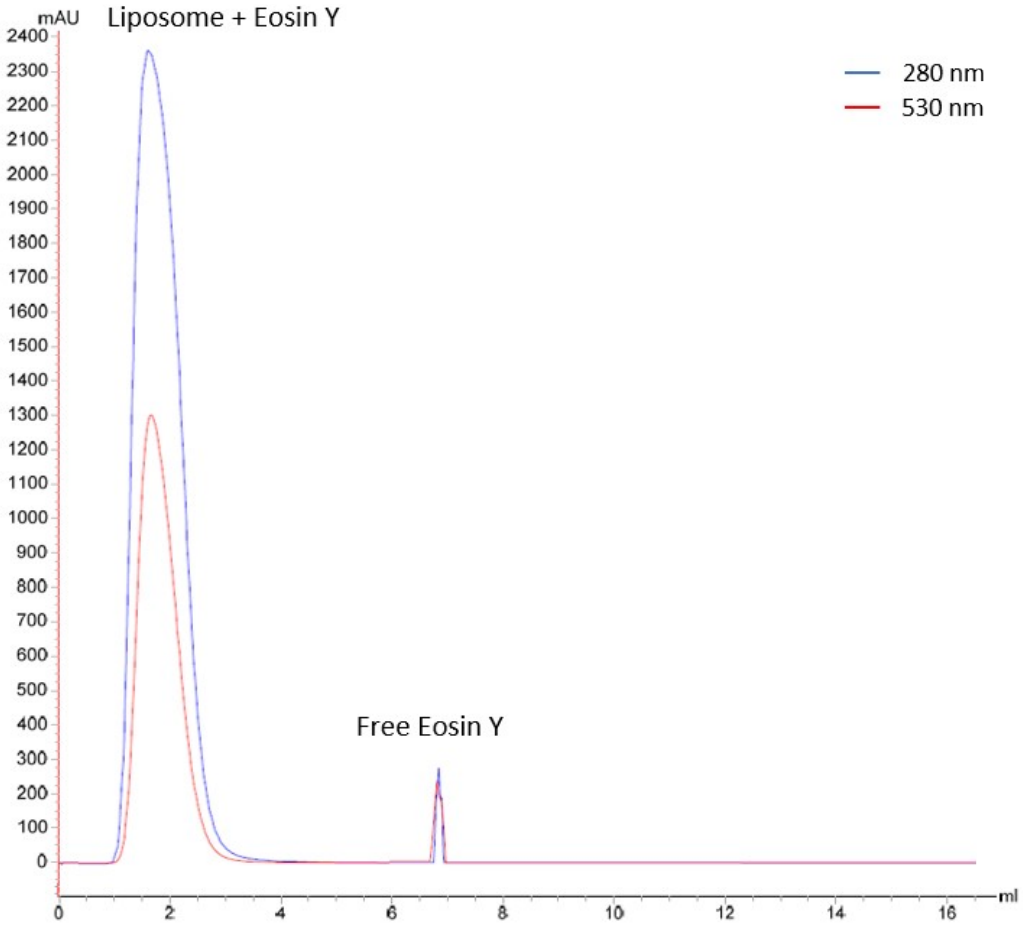


Figure S18: FPLC chromatogram of liposome encapsulating eosin Y (7 days)

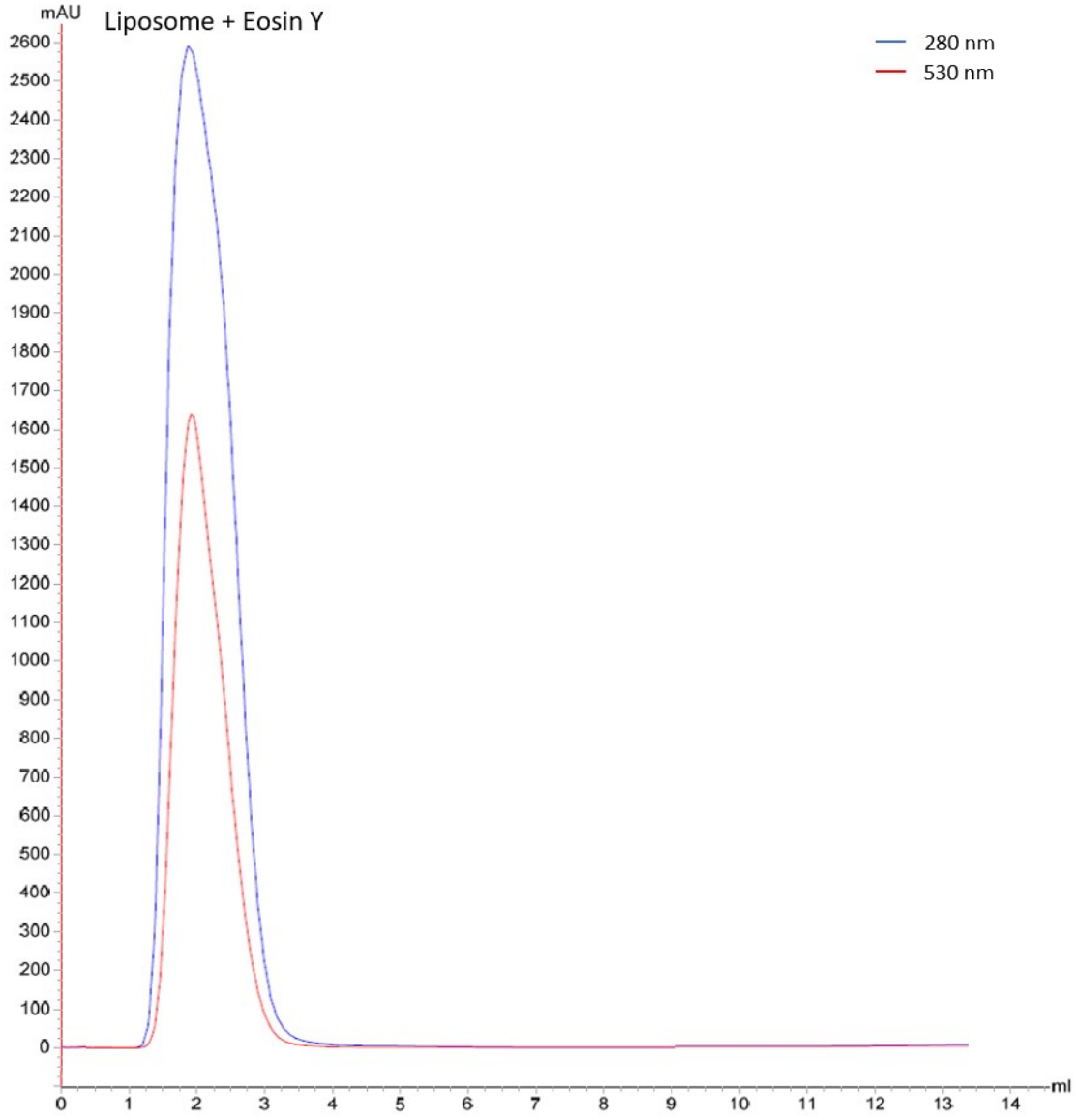


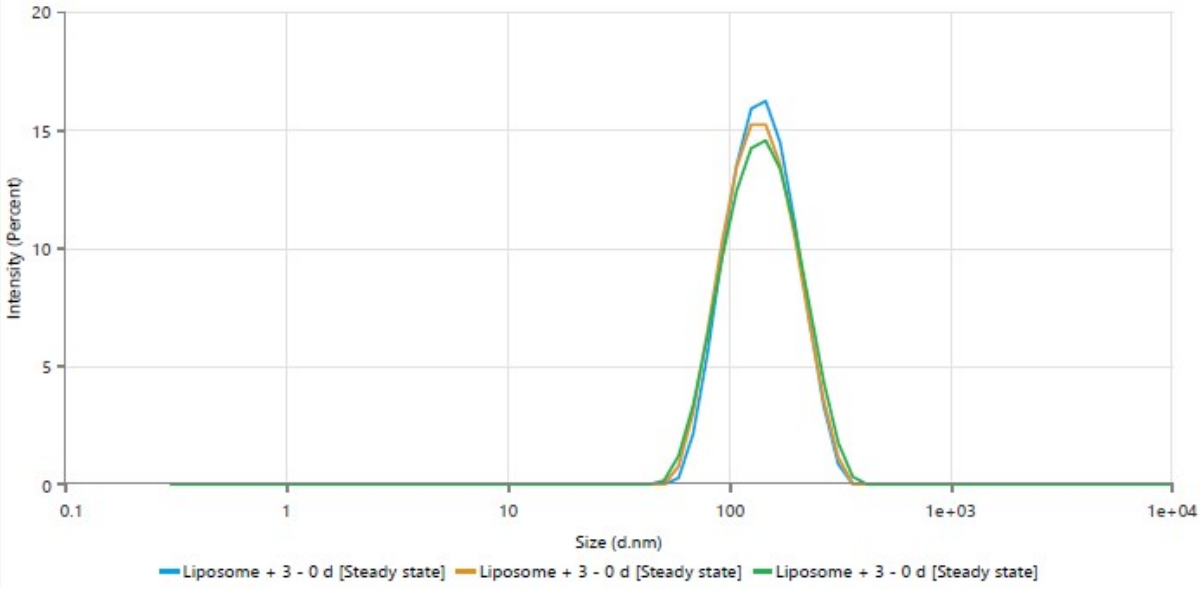
Figure S19: DLS report of liposome encapsulating phthalocyanine 3 (0 days)

Size Distribution Report

Statistics Table

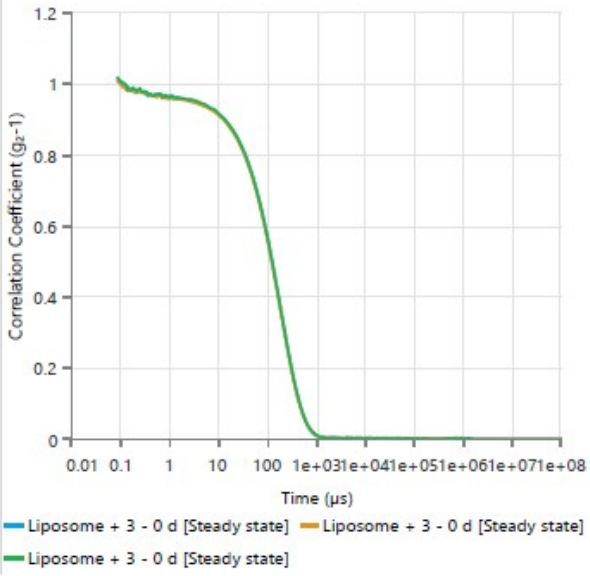
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	129.1	1.778	1.377	127.2	130.8
Polydispersity Index (PI)	0.1193	0.01096	9.188	0.1067	0.1257
Temperature (°C)	25	0	0	25	25
Cuvette Position (mm)	1.24	0	0	1.24	1.24
Attenuator	3	0	0	3	3
Mean Count Rate (kcps)	270.5	1.308	0.4835	269.2	271.8
Peak 1 Area by Intensity ordered by area (%)	100	0	0	100	100
Peak 1 Mean by Intensity ordered by area (nm)	147.4	1.827	1.239	145.7	149.3
Peak 1 Width by Intensity ordered by area (nm)	53.12	3.469	6.53	50.07	56.9
Peak 1 Area by Number ordered by area (%)	100	0	0	100	100
Peak 1 Mean by Number ordered by area (nm)	86.88	5.242	6.034	81.75	92.22
Peak 1 Width by Number ordered by area (nm)	29.88	0.705	2.359	29.32	30.67
Peak 1 Area by Number ordered by size (%)	100	0	0	100	100
Peak 1 Mean by Number ordered by size (nm)	86.88	5.242	6.034	81.75	92.22
Peak 1 Width by Number ordered by size (nm)	29.88	0.705	2.359	29.32	30.67

Size Distribution by Intensity

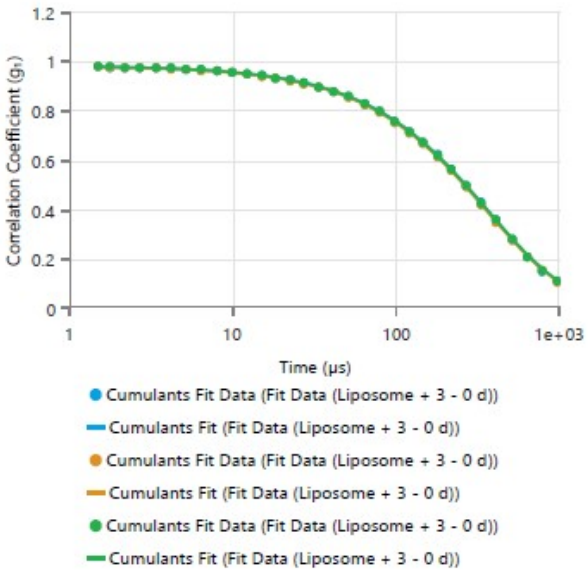


Size Distribution Report

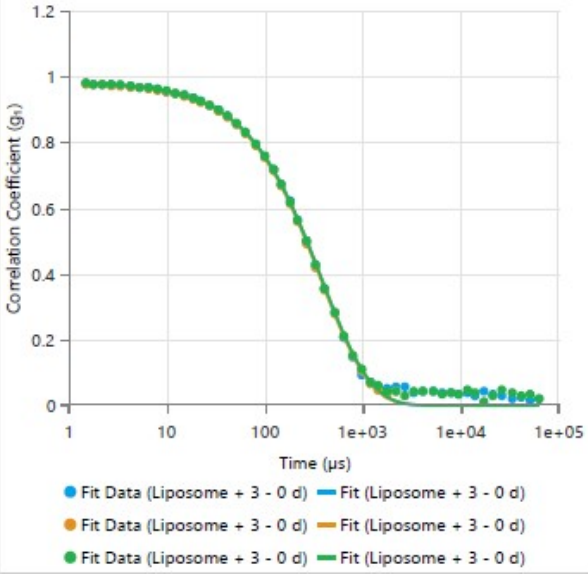
Correlogram



Cumulants Fit



Distribution Fit



Size Distribution by Number

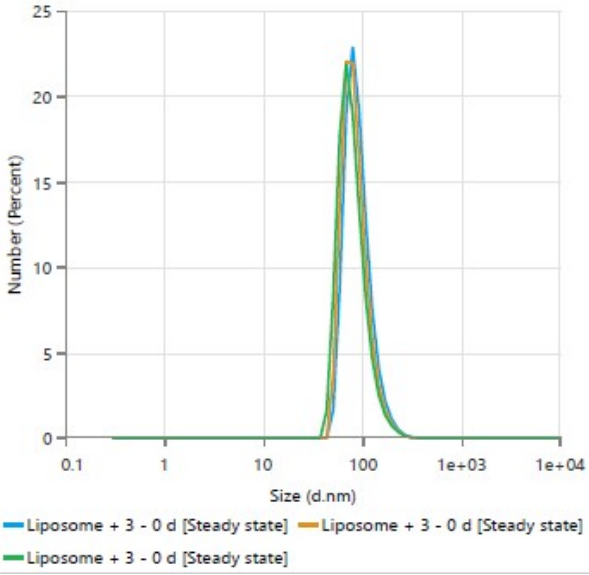
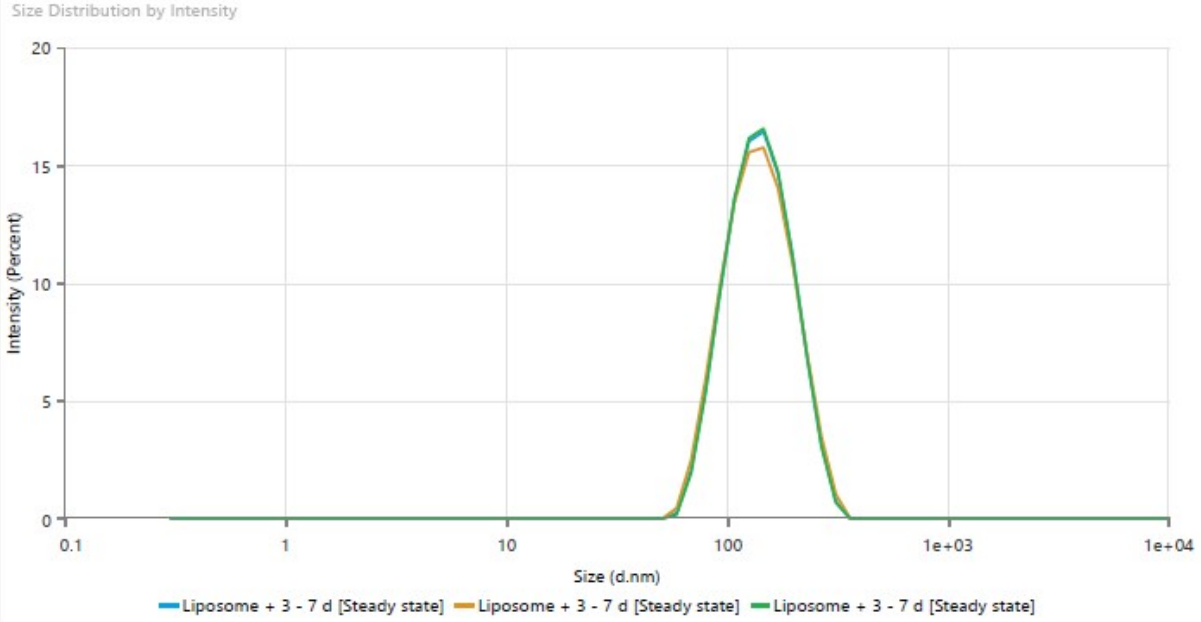


Figure S20: DLS report of liposome encapsulating phthalocyanine 3 (7 days)

Size Distribution Report

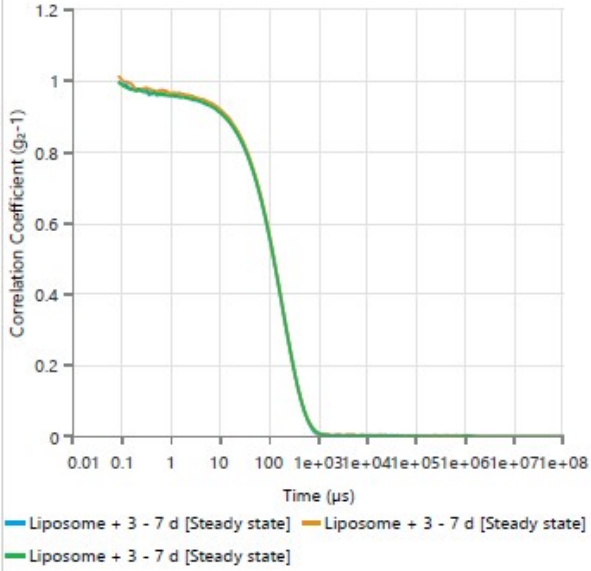
Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	130.2	0.2936	0.2255	129.9	130.4
Polydispersity Index (PI)	0.1074	0.001237	1.152	0.1059	0.1082
Temperature (°C)	25	0	0	25	25
Cuvette Position (mm)	1.24	0	0	1.24	1.24
Attenuator	3	0	0	3	3
Mean Count Rate (kcps)	322	1.024	0.3181	320.8	322.6
Peak 1 Area by Intensity ordered by area (%)	100	0	0	100	100
Peak 1 Mean by Intensity ordered by area (nm)	147.1	0.07711	0.05242	147	147.2
Peak 1 Width by Intensity ordered by area (nm)	49.9	1.248	2.5	49.01	51.33
Peak 1 Area by Number ordered by area (%)	100	0	0	100	100
Peak 1 Mean by Number ordered by area (nm)	92.03	2.027	2.202	89.8	93.74
Peak 1 Width by Number ordered by area (nm)	30.61	0.3145	1.028	30.25	30.8
Peak 1 Area by Number ordered by size (%)	100	0	0	100	100
Peak 1 Mean by Number ordered by size (nm)	92.03	2.027	2.202	89.8	93.74
Peak 1 Width by Number ordered by size (nm)	30.61	0.3145	1.028	30.25	30.8

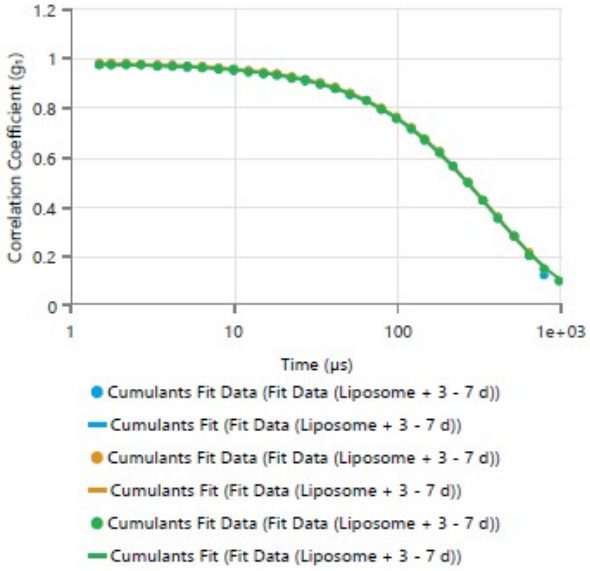


Size Distribution Report

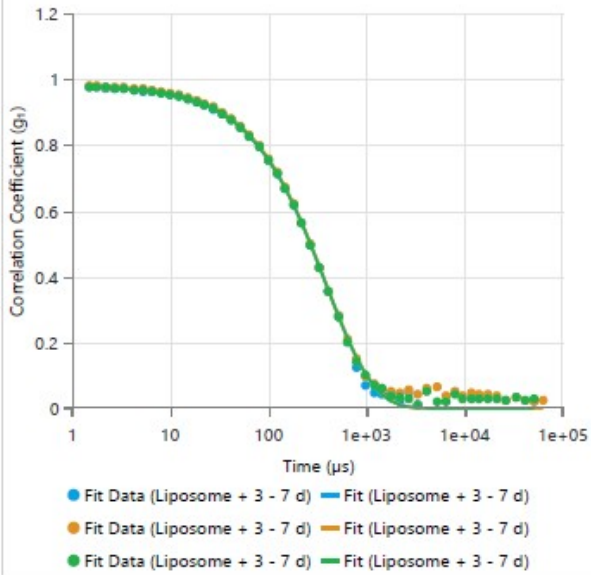
Correlogram



Cumulants Fit



Distribution Fit



Size Distribution by Number

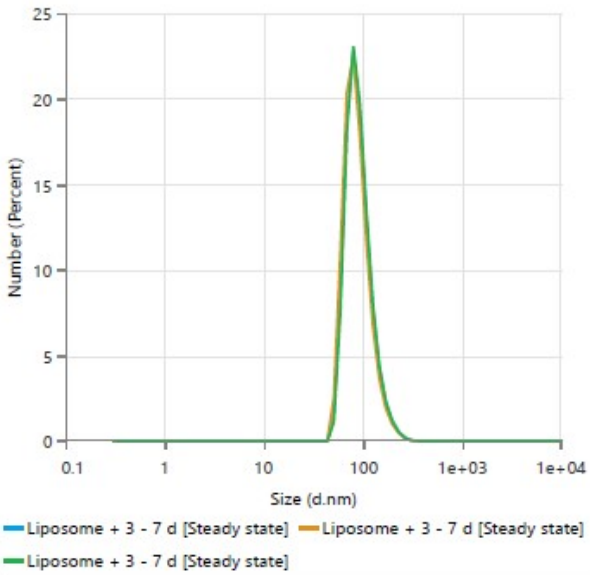
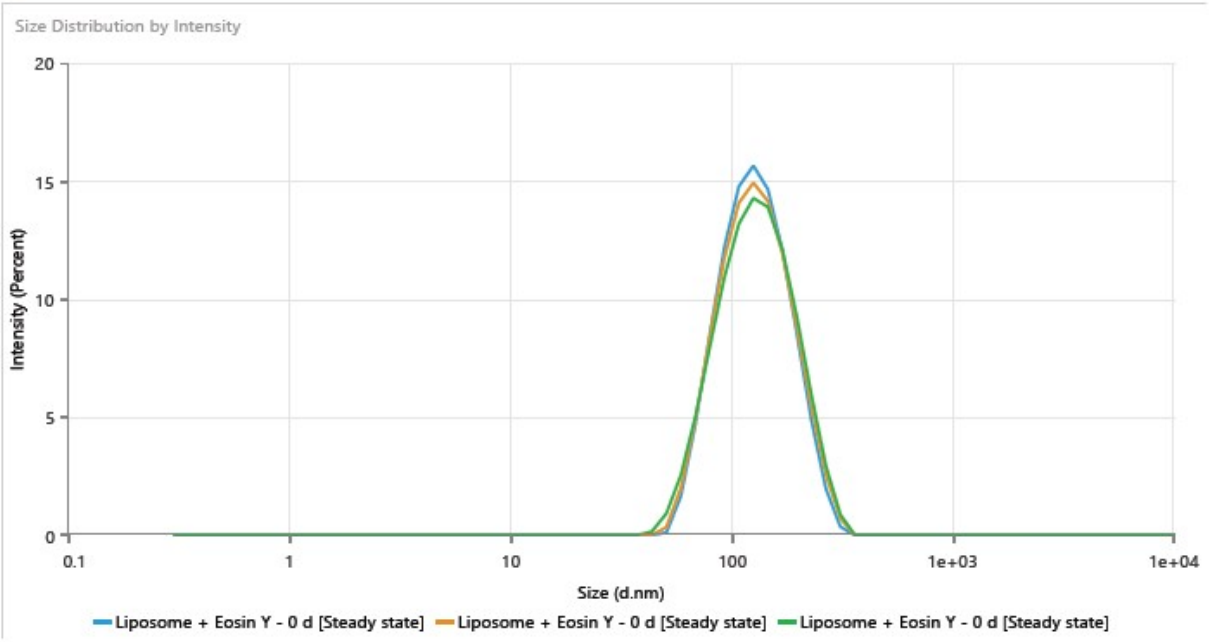


Figure S21: DLS report of liposome encapsulating Eosin Y (0 days)

Size Distribution Report

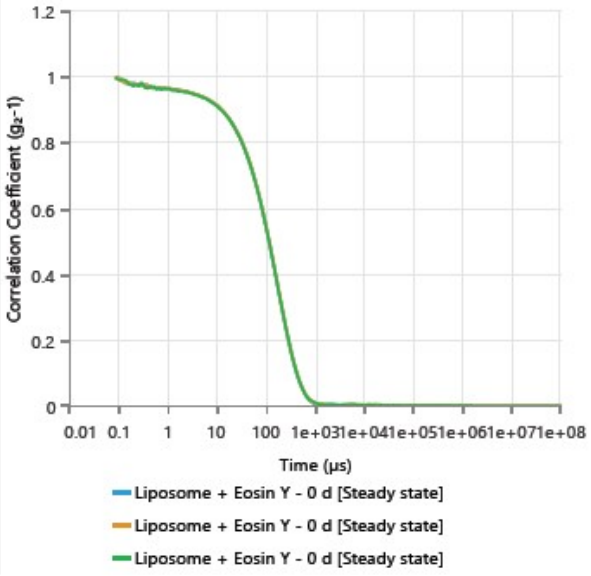
Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	119.4	0.4869	0.4076	119	120
Polydispersity Index (PI)	0.115	0.008742	7.603	0.1051	0.1215
Temperature (°C)	25	0	0	25	25
Cuvette Position (mm)	4.64	0	0	4.64	4.64
Attenuator	4	0	0	4	4
Mean Count Rate (kcps)	340.7	1.236	0.3628	339.5	342
Peak 1 Area by Intensity ordered by area (%)	100	0	0	100	100
Peak 1 Mean by Intensity ordered by area (nm)	136	1.653	1.215	134.3	137.5
Peak 1 Width by Intensity ordered by area (nm)	50.66	2.652	5.234	47.88	53.16
Peak 1 Area by Number ordered by area (%)	100	0	0	100	100
Peak 1 Mean by Number ordered by area (nm)	75.84	5.163	6.808	70.15	80.22
Peak 1 Width by Number ordered by area (nm)	25.8	0.1753	0.6792	25.6	25.92
Peak 1 Area by Number ordered by size (%)	100	0	0	100	100
Peak 1 Mean by Number ordered by size (nm)	75.84	5.163	6.808	70.15	80.22
Peak 1 Width by Number ordered by size (nm)	25.8	0.1753	0.6792	25.6	25.92

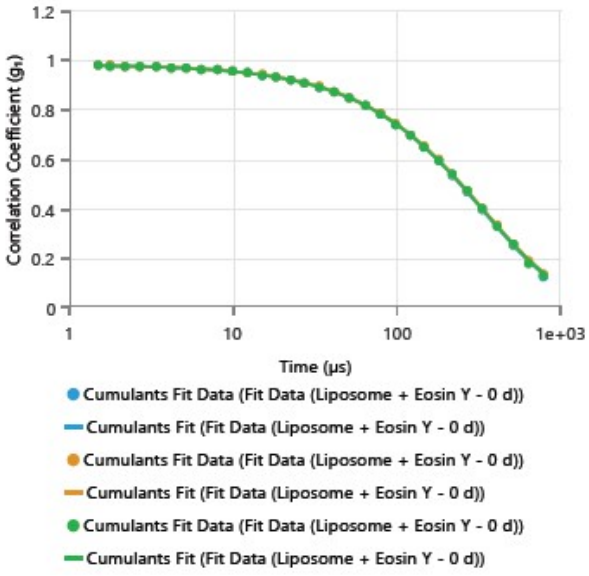


Size Distribution Report

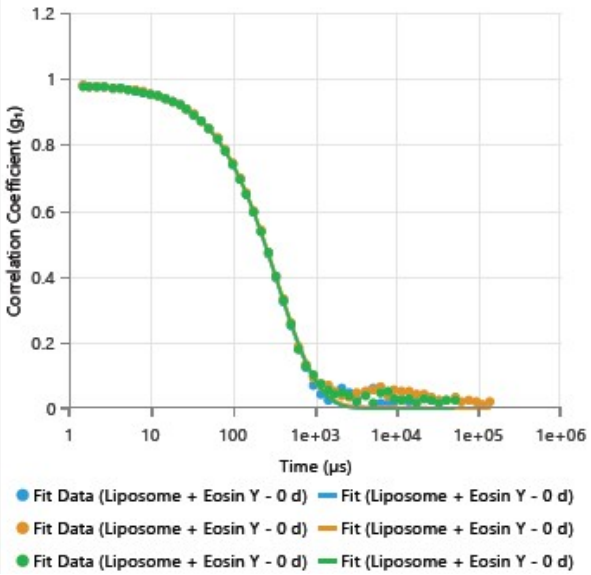
Correlogram



Cumulants Fit



Distribution Fit



Size Distribution by Number

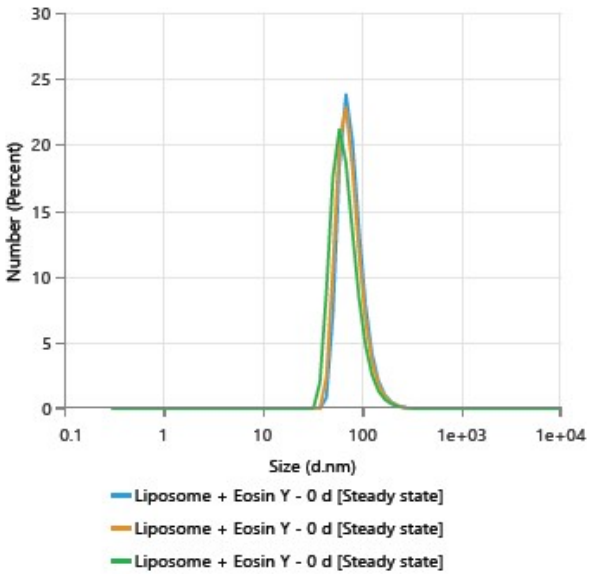
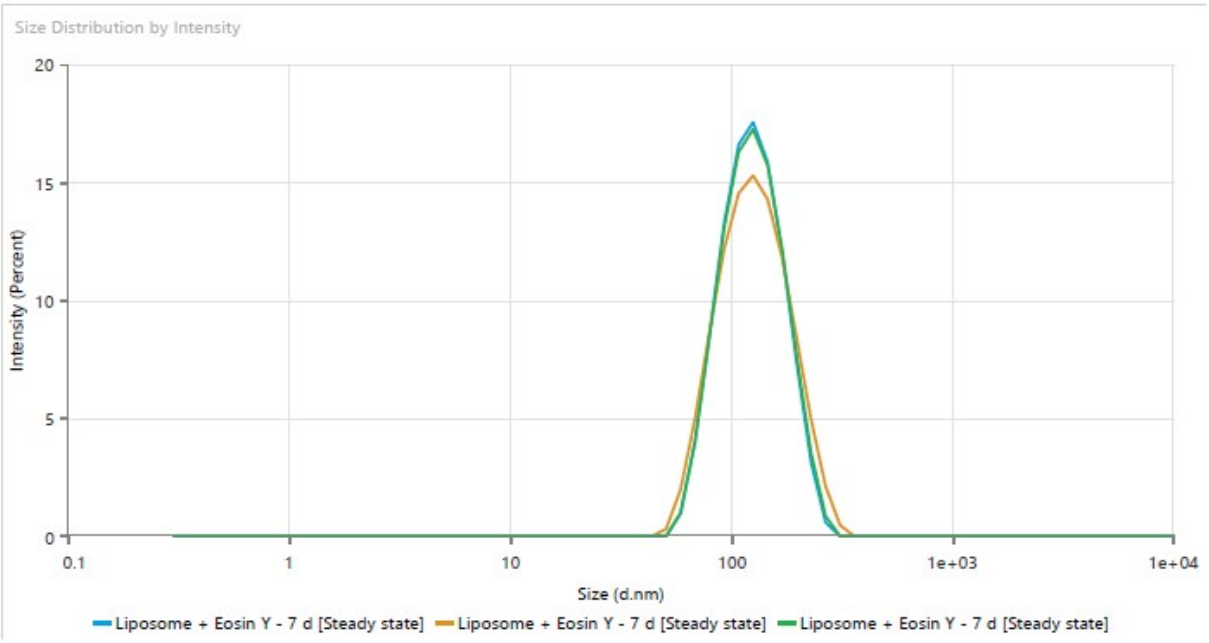


Figure S22: DLS report of liposome encapsulating Eosin Y (7 days)

Size Distribution Report

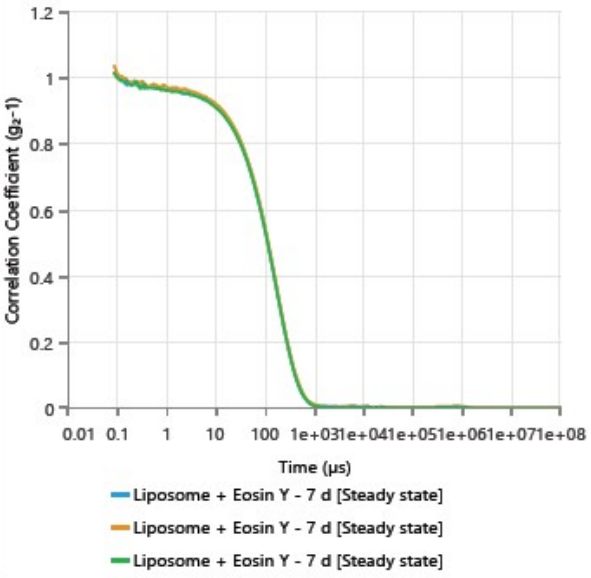
Statistics Table

Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	117.2	0.5194	0.443	116.6	117.5
Polydispersity Index (PI)	0.09977	0.01919	19.23	0.07868	0.1162
Temperature (°C)	25	0	0	25	25
Cuvette Position (mm)	4.64	0	0	4.64	4.64
Attenuator	4	0	0	4	4
Mean Count Rate (kcps)	282.7	0.7491	0.265	281.9	283.2
Peak 1 Area by Intensity ordered by area (%)	100	0	0	100	100
Peak 1 Mean by Intensity ordered by area (nm)	131.4	2.312	1.76	129.4	133.9
Peak 1 Width by Intensity ordered by area (nm)	44.01	4.386	9.967	40.9	49.02
Peak 1 Area by Number ordered by area (%)	100	0	0	100	100
Peak 1 Mean by Number ordered by area (nm)	82.14	4.067	4.951	77.44	84.51
Peak 1 Width by Number ordered by area (nm)	25.66	0.1241	0.4836	25.59	25.81
Peak 1 Area by Number ordered by size (%)	100	0	0	100	100
Peak 1 Mean by Number ordered by size (nm)	82.14	4.067	4.951	77.44	84.51
Peak 1 Width by Number ordered by size (nm)	25.66	0.1241	0.4836	25.59	25.81

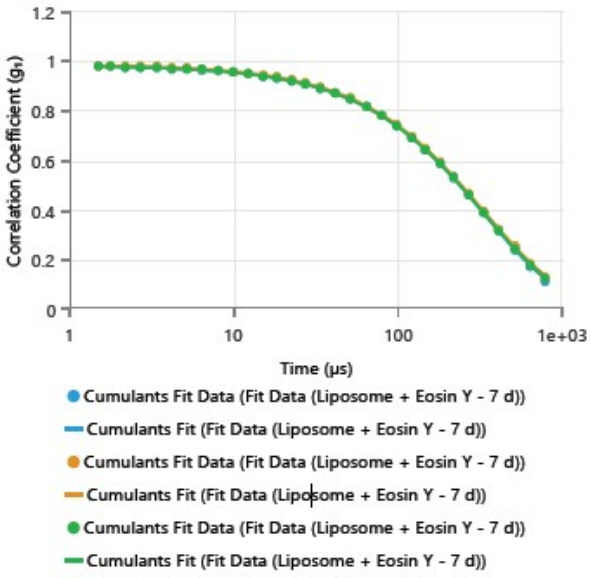


Size Distribution Report

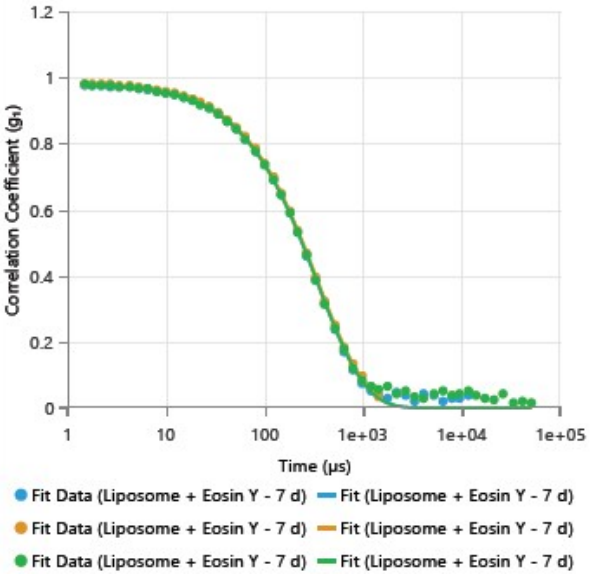
Correlogram



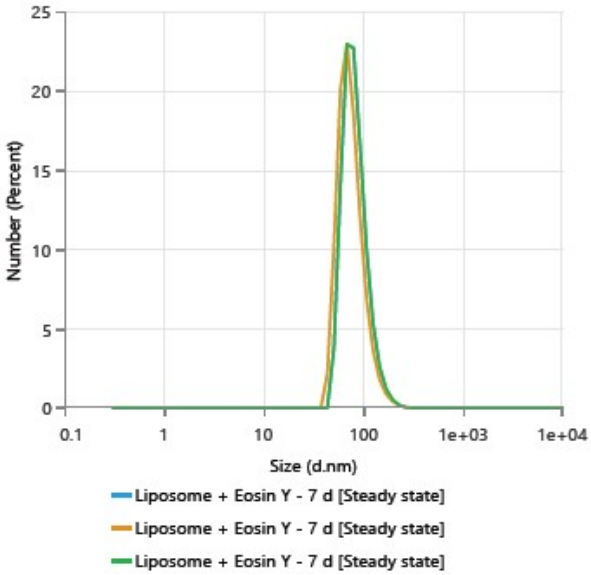
Cumulants Fit



Distribution Fit

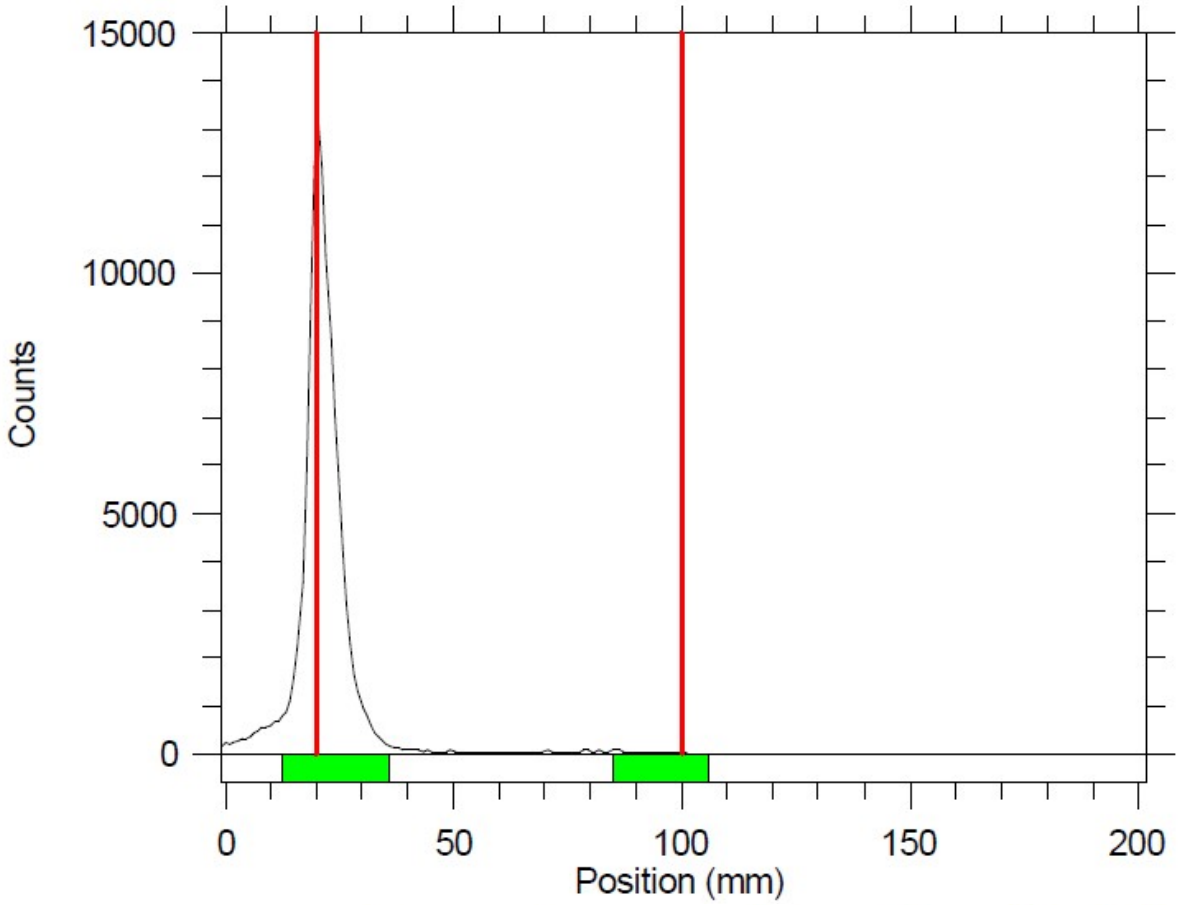


Size Distribution by Number



8. Radiolabeling

Figure S23: radio-TLC performed after the radiolabeling of the liposome NODAGA



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	12.5	36.3	21.6	0.021	110809.0	110809.0	91.54	99.24
Rgn 2	84.7	105.8	91.8	0.897	850.0	850.0	0.70	0.76
2 Peaks					111659.0	111659.0	92.24	100.00

9. Cherenkov Luminescence Experiments

Figure S24: CLI performed upon mixing liposome/liposome encapsulating Eosin Y/Eosin Y with [^{68}Ga]-$GaCl_3$ (1.5 MBq, values measured from ROI) in open filter mode

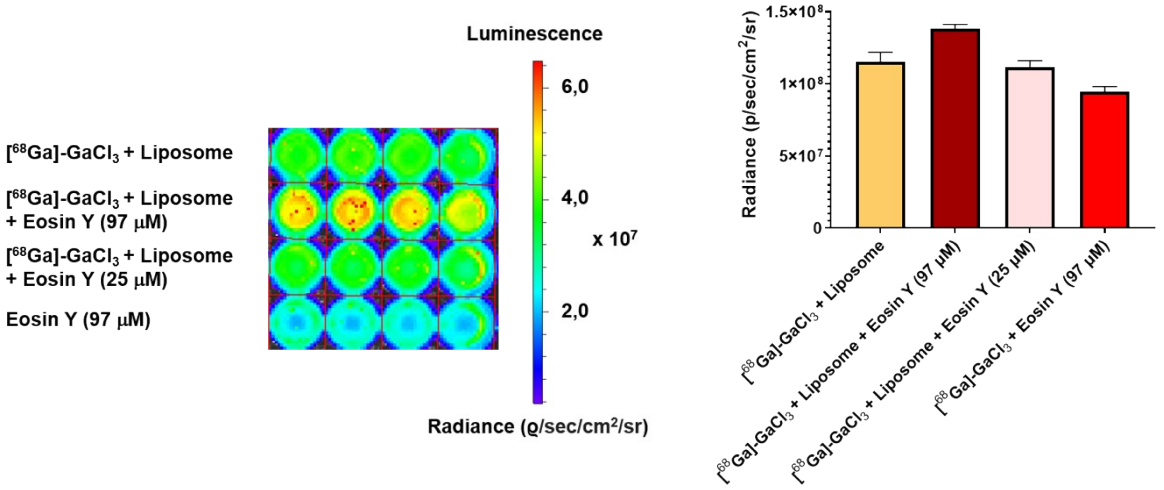


Figure S25: CLI performed upon mixing liposome/liposome encapsulating compound 3/compound 3 with [^{68}Ga]-$GaCl_3$ (1.5 MBq, values measured from ROI) in open filter mode

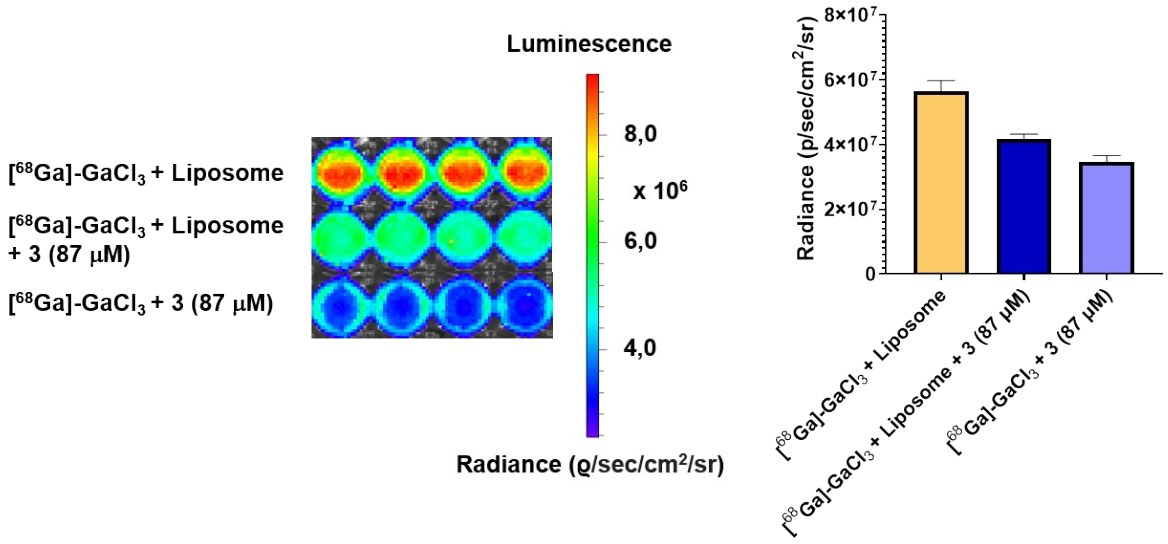


Figure S26: CLI performed upon mixing liposome/liposome encapsulating compound 3/compound 3 with [^{68}Ga]-$GaCl_3$ (1.5 MBq, values measured from ROI) when using the 670 nm filter

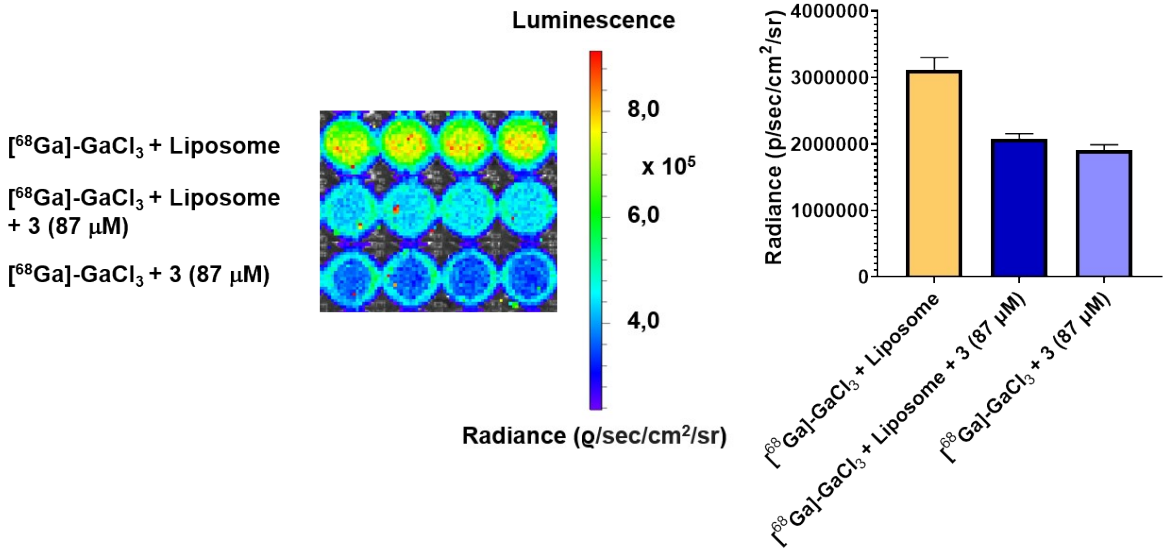


Figure S27: CLI performed upon comparing liposome with solvents of comparable refractive index, in the presence of [^{68}Ga]-$GaCl_3$ (1.5 MBq, values measured from ROI). Top: Diagram bar labelled with a) circles: experiment performed in pure solvent, b) rectangle: experiment performed with addition of 10% solvent. Bottom: 96-well plate image of the corresponding diagram. Doted lines: when precipitation was suspected.

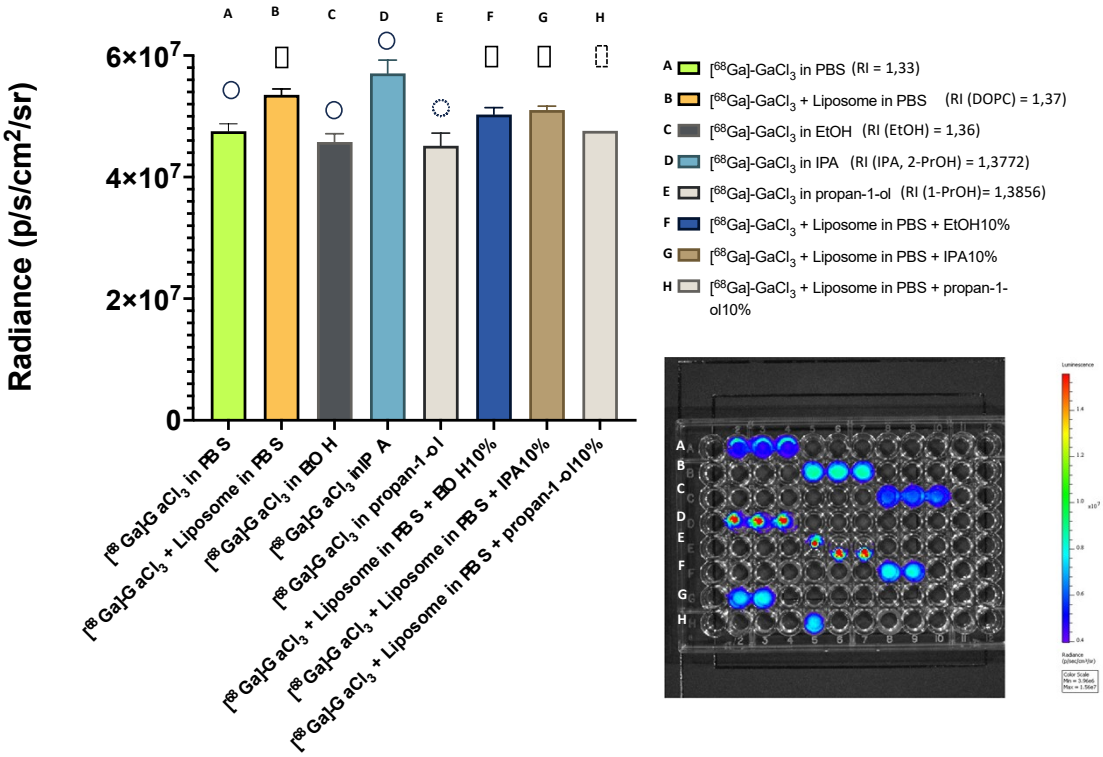


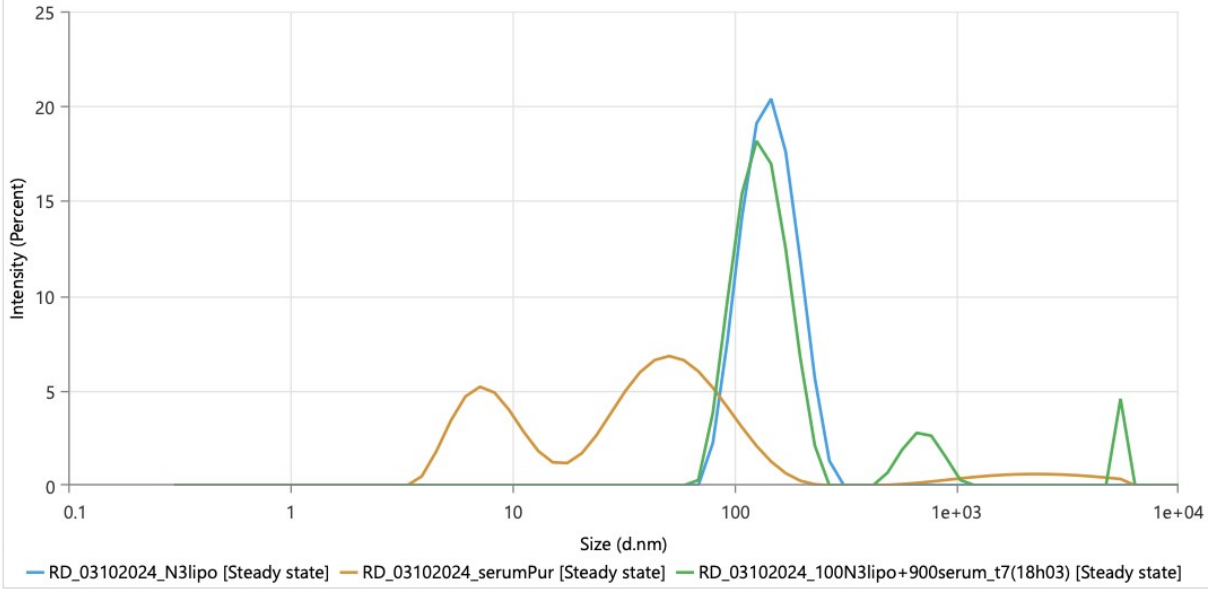
Figure S28-1: DLS report of a liposome suspension in a PBS/ Fetal Calf Serum mixture (1:1 vol.) at t1 = 6 h. Blue: liposome suspension in PBS, Orange: pure serum, Green: liposome suspension in PBS/ serum (1:1 vol.).

Size Distribution Report

Statistics Table

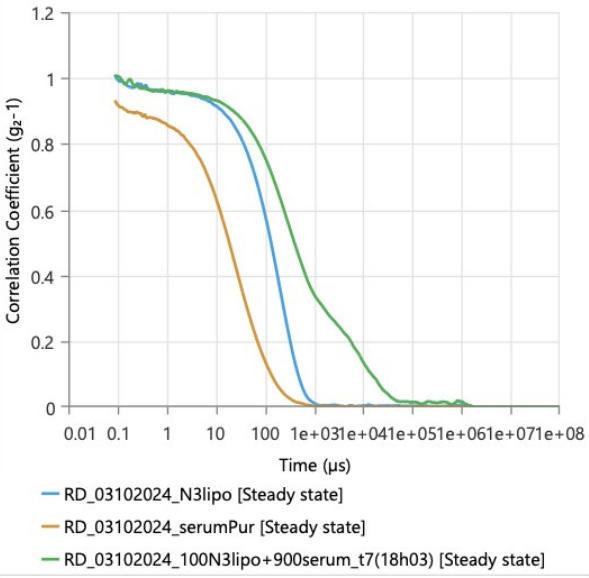
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	171.9	172.3	100.3	19.64	359
Polydispersity Index (PI)	0.5422	0.4267	78.7	0.05215	0.8316
Temperature (°C)	25	0	0	25	25
Cuvette Position (mm)	2.24	2.088	93.22	0.84	4.64
Attenuator	4.667	1.155	24.74	4	6
Mean Count Rate (kcps)	264.9	38.87	14.67	222.8	299.4
Peak 1 Area by Intensity ordered by area (%)	82.65	19.07	23.07	62.24	100
Peak 1 Mean by Intensity ordered by area (nm)	114.4	47.39	41.42	60.19	147.9
Peak 1 Width by Intensity ordered by area (nm)	35.95	3.676	10.23	32.78	39.98
Peak 2 Area by Intensity ordered by area (%)	20.43	15.14	74.13	9.721	31.14
Peak 2 Mean by Intensity ordered by area (nm)	357.6	493.7	138.1	8.504	706.7
Peak 2 Width by Intensity ordered by area (nm)	67.23	90.52	134.6	3.222	131.2
Peak 3 Area by Intensity ordered by area (%)	5.594	1.456	26.04	4.564	6.624
Peak 3 Mean by Intensity ordered by area (nm)	3954	2141	54.15	2440	5468
Peak 3 Width by Intensity ordered by area (nm)	660.4	934	141.4	6.104E-05	1321
Peak 1 Area by Number ordered by area (%)	99.96	0.06611	0.06614	99.89	100
Peak 1 Mean by Number ordered by area (nm)	72.74	58.69	80.69	5.141	110.7

Size Distribution by Intensity

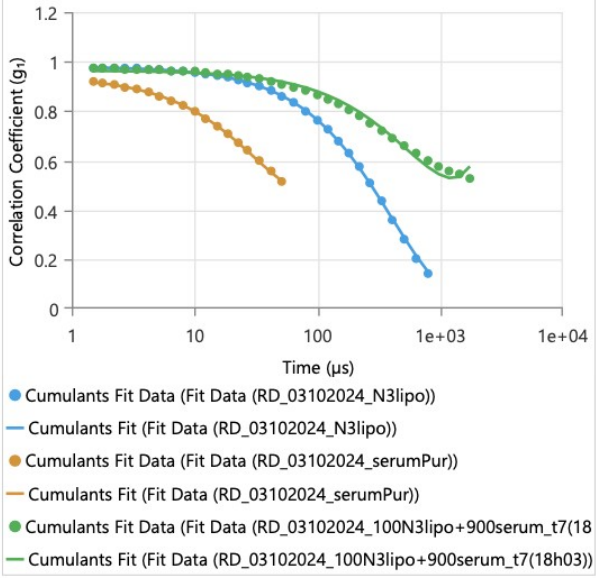


Size Distribution Report

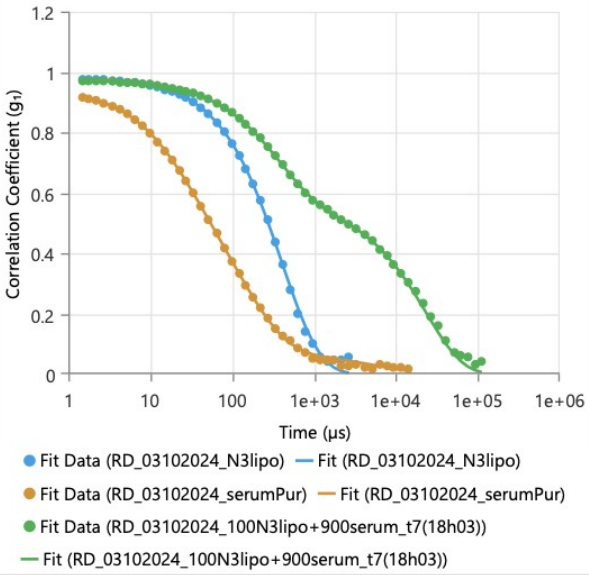
Correlogram



Cumulants Fit



Distribution Fit



Size Distribution by Number

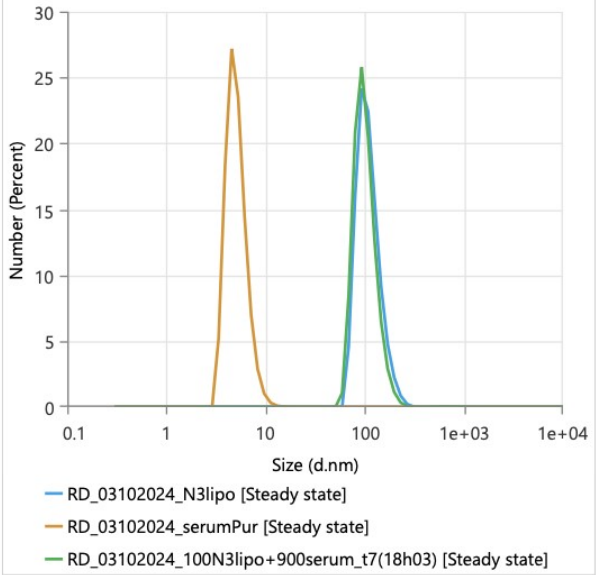


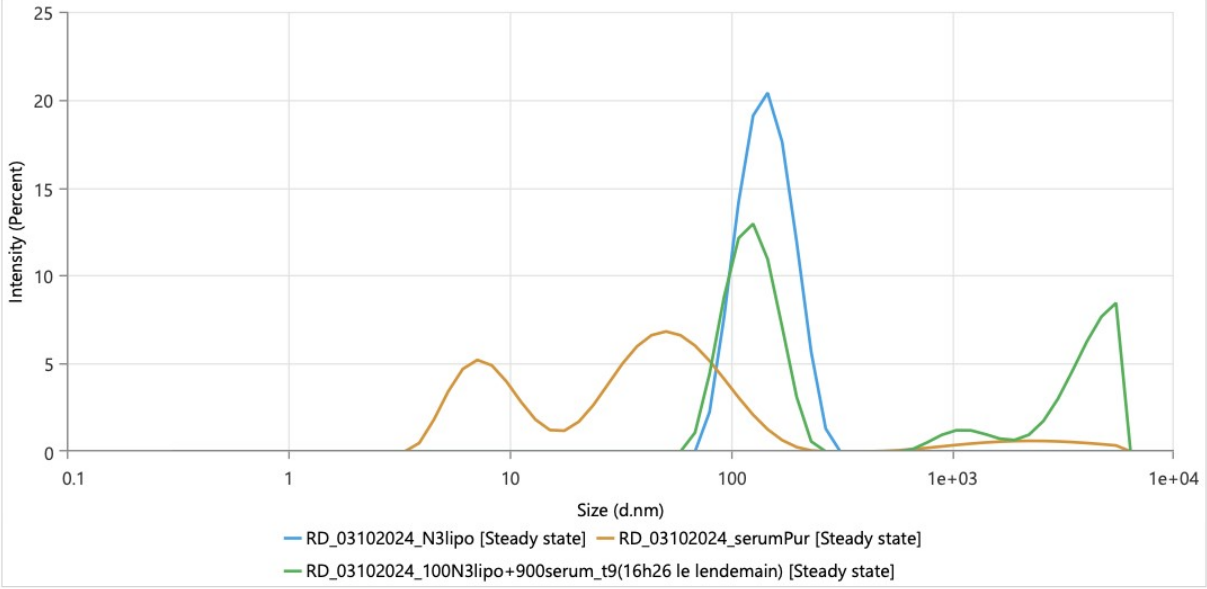
Figure S28-2: DLS report of a liposome suspension in a PBS/ Fetal Calf Serum mixture (1:1 vol.) at t2 = 22 h. Blue: liposome suspension in PBS, Orange: pure serum, Green: liposome suspension in PBS/ serum (1:1 vol.).

Size Distribution Report

Statistics Table

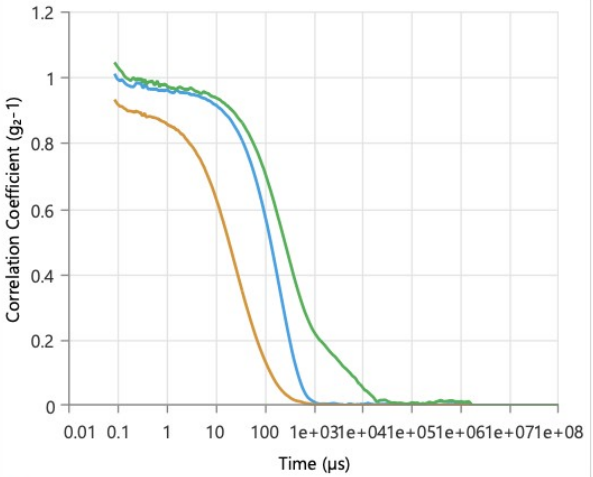
Name	Mean	Standard Deviation	RSD	Minimum	Maximum
Z-Average (nm)	127.6	103.5	81.13	19.64	226
Polydispersity Index (PI)	0.5079	0.3947	77.72	0.05215	0.7428
Temperature (°C)	25	0	0	25	25
Cuvette Position (mm)	2.24	2.088	93.22	0.84	4.64
Attenuator	4.667	1.155	24.74	4	6
Mean Count Rate (kcps)	253.7	57.57	22.69	189	299.4
Peak 1 Area by Intensity ordered by area (%)	74.29	22.28	30	60.62	100
Peak 1 Mean by Intensity ordered by area (nm)	111.5	45.72	41	60.19	147.9
Peak 1 Width by Intensity ordered by area (nm)	35.26	4.095	11.61	32.78	39.98
Peak 2 Area by Intensity ordered by area (%)	32.11	1.371	4.271	31.14	33.07
Peak 2 Mean by Intensity ordered by area (nm)	2110	2973	140.9	8.504	4212
Peak 2 Width by Intensity ordered by area (nm)	508.6	714.7	140.5	3.222	1014
Peak 3 Area by Intensity ordered by area (%)	6.466	0.223	3.449	6.308	6.624
Peak 3 Mean by Intensity ordered by area (nm)	1836	854	46.51	1232	2440
Peak 3 Width by Intensity ordered by area (nm)	831.8	691.7	83.15	342.7	1321
Peak 1 Area by Number ordered by area (%)	99.99	0.01794	0.01795	99.97	100
Peak 1 Mean by Number ordered by area (nm)	70.1	56.84	81.08	5.141	110.7

Size Distribution by Intensity

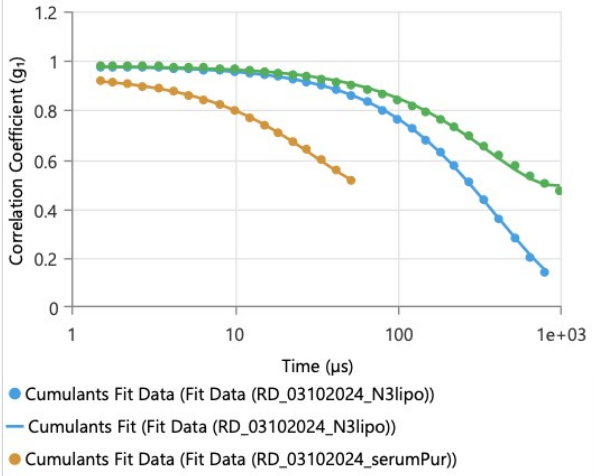


Size Distribution Report

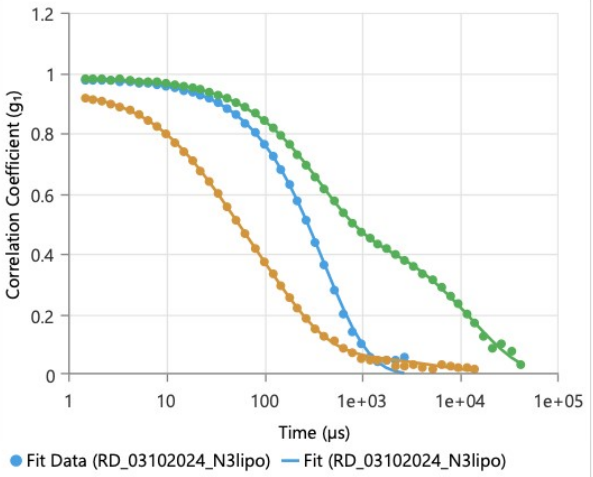
Correlogram



Cumulants Fit



Distribution Fit



Size Distribution by Number