SUPPLEMENTARY INFORMATION

Multimerized Self-assembled Caged *Two-in-One* siRNA Nanoparticles for Photomodulation of RNAi-Induced Gene Silencing

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Table S1. The sequences of native and chemical-modified oligonucleotides used in this study

Target	Name	Sequence (5′)	Calc.	Meas.
GFP	SG	GAA CGG CAU CAA GGU GAA CTT	6755	6756.7
	AG	GUU CAC CUU GAU GCC GUU CTT	6561	6562.2
	Chol-*p*-SG-*p*-SS	Chol-*p*-GAA CGG CAU CAA GGU GAA CTT-*p*-SS	8326	8327.0
	Chol-*p*-AG-*p*-SS	Chol-*p*-GUU CAC CUU GAU GCC GUU CTT-*p*-SS	8131	8131.5
	Chol-*p*-SG-*p*-NH_2	Chol-*p*-GAA CGG CAU CAA GGU GAA CTT-*p*-NH_2	7903	7904.8
	Chol-*p*-AG-*p*-NH_2	Chol-*p*-GUU CAC CUU GAU GCC GUU CTT-*p*-NH_2	7708	7709.5
Eg5	SE	CAA CAA GGA UGA AGU CUA UTT	6701	6701.6
	AE	AUA GAC UUC AUC CUU GUU GTT	6568	6570.3
	Chol-*p*-SE-*p*-SS	Chol-*p*-CAA CAA GGA UGA AGU CUA UTT-*p*-SS	8271	8274.4
	Chol-*p*-AE-*p*-SS	Chol-*p*-AUA GAC UUC AUC CUU GUU GTT-*p*-SS	8139	8140.9

S, sense strand RNA; A, antisense strand RNA; G, GFP; E, Eg5; Chol, cholesterol; *p*, photolinker; SS, thiol modifier C6 S-S; NH_2, 3′-Amino-Modifier C7

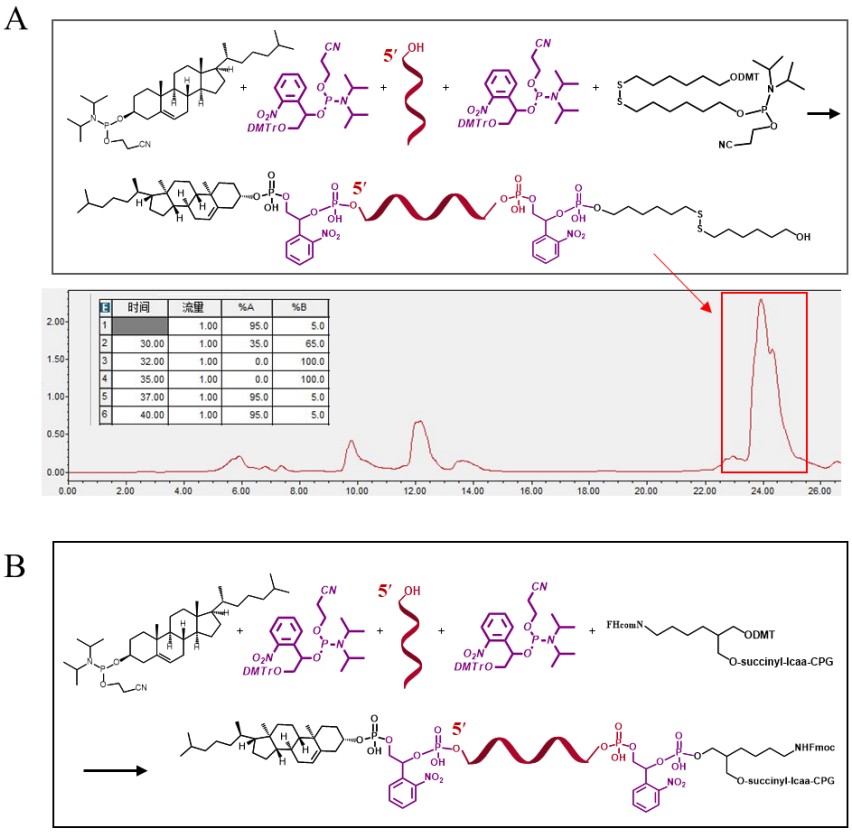
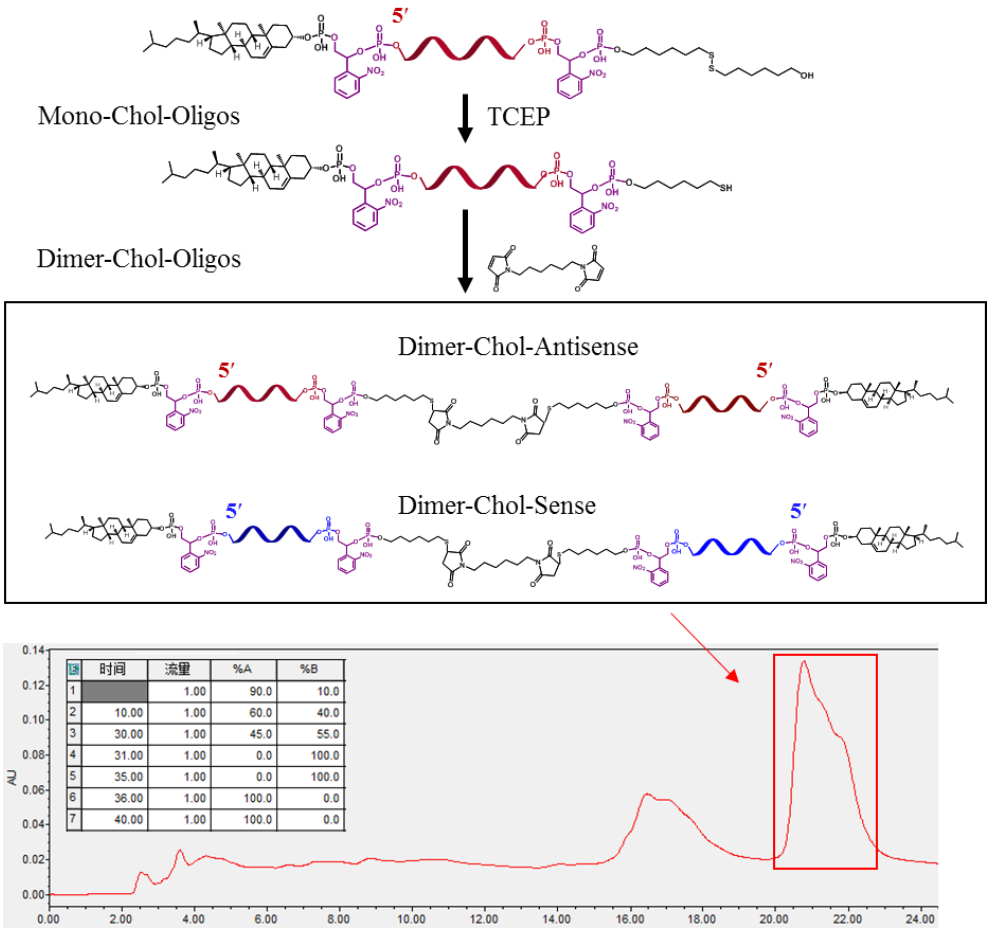


Figure S1. Synthesis and HPLC purification of thiol-modified caged oligonucleotides with 5′ terminal cholesterol modifictation (A) and amine-modified caged oligonucleotides with 5′ terminal cholesterol modifictation (B).



	时间	流量	%A	%B
1		1.00	90.0	10.0
2	10.00	1.00	60.0	40.0
3	30.00	1.00	45.0	55.0
4	31.00	1.00	0.0	100.0
5	35.00	1.00	0.0	100.0
6	36.00	1.00	100.0	0.0
7	40.00	1.00	100.0	0.0

Figure S2. Synthesis and HPLC purification of single-strand caged sense dimer (Dimer-Chol-Sense) and caged antisense dimer (Dimer-Chol-Antisense) with cholesterol modification.

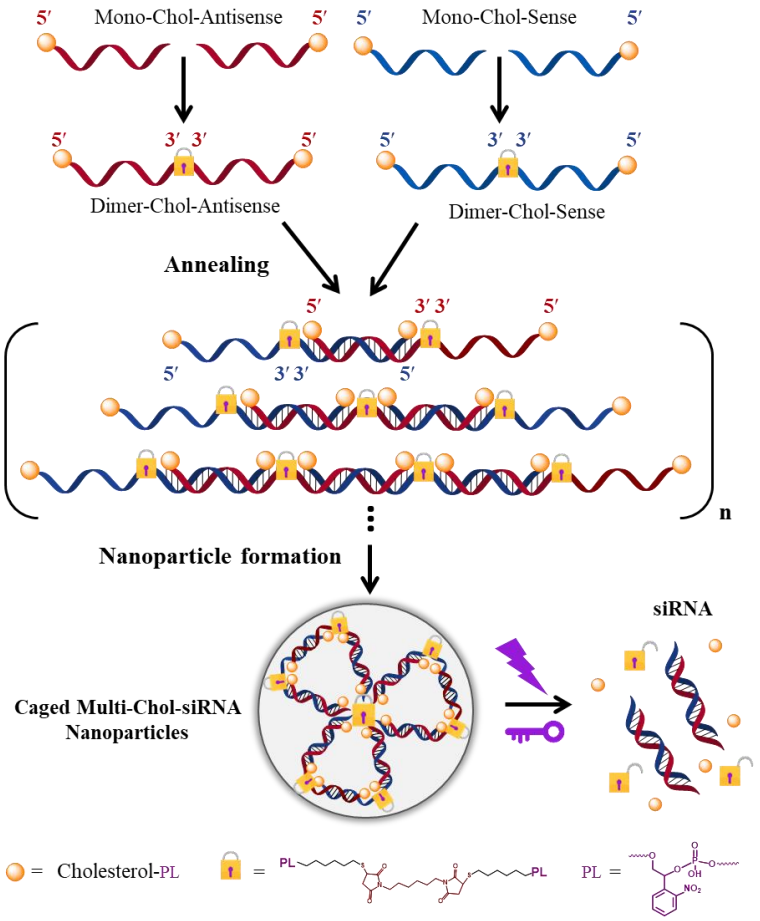


Figure S3. Rational design and synthesis of caged Multi-Chol-siRNA self-assembled nanoparticles

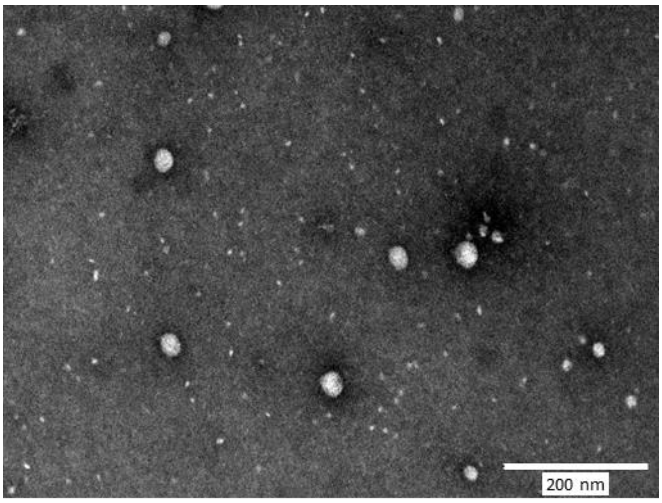


Figure S4. TEM image of caged Multi-Chol-siRNA self-assembled nanoparticles upon UV light irradiation

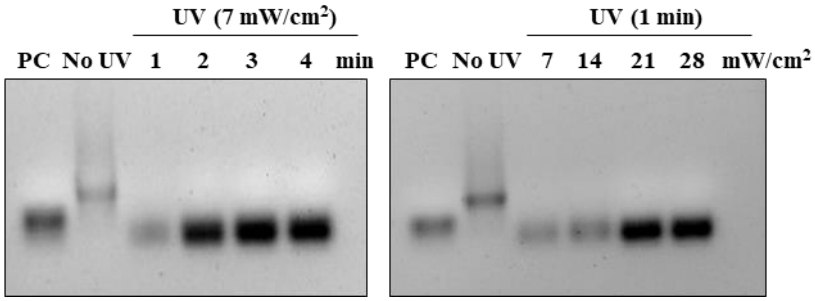


Figure S5. Gel-shift assay for evaluation of photosensitivity of Multi-Chol-siRNA self-assembled nanoparticles before or after UV light irradiation

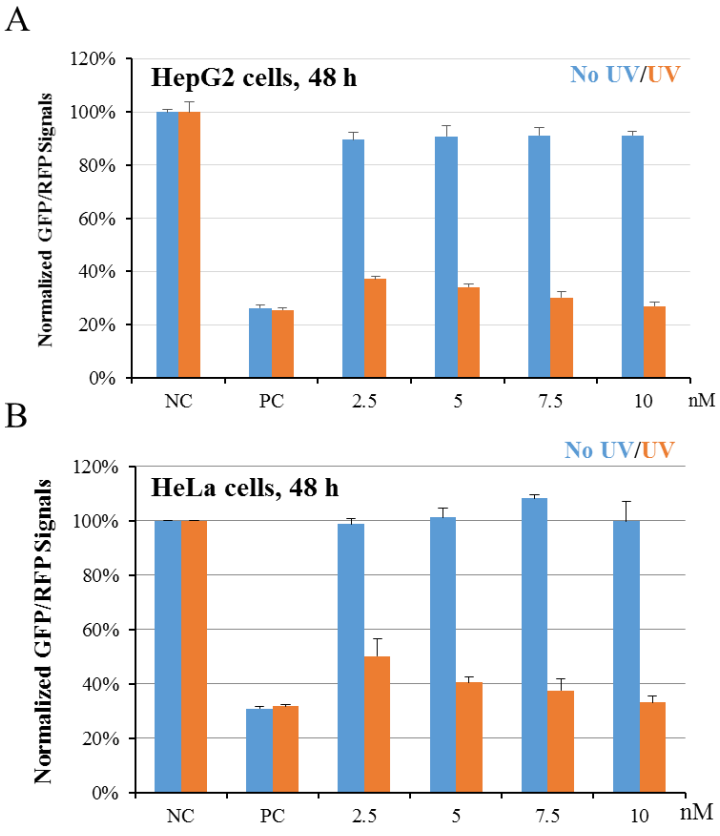


Figure S6. Photochemical regulation of GFP gene expression with caged Multi-Chol-siGFP self-assembled nanoparticles in HepG2 cells (A) and HeLa cells (B) for 48 h incubation quantitated by flow cytometry.

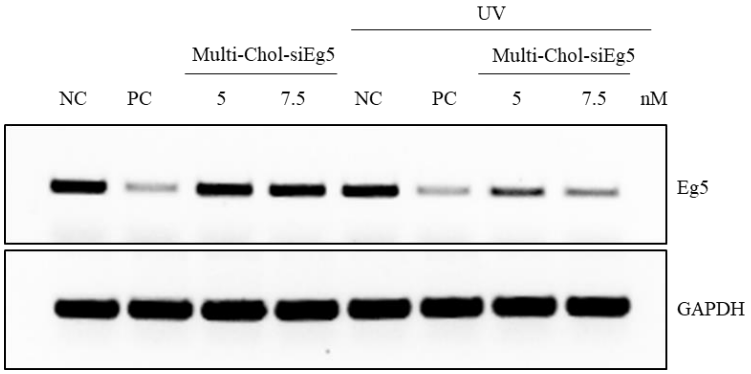


Figure S7. Photochemical regulation of Eg5 mRNA levels using caged Multi-Chol-siEg5 self-assembled nanoparticles determined by RT-PCR.

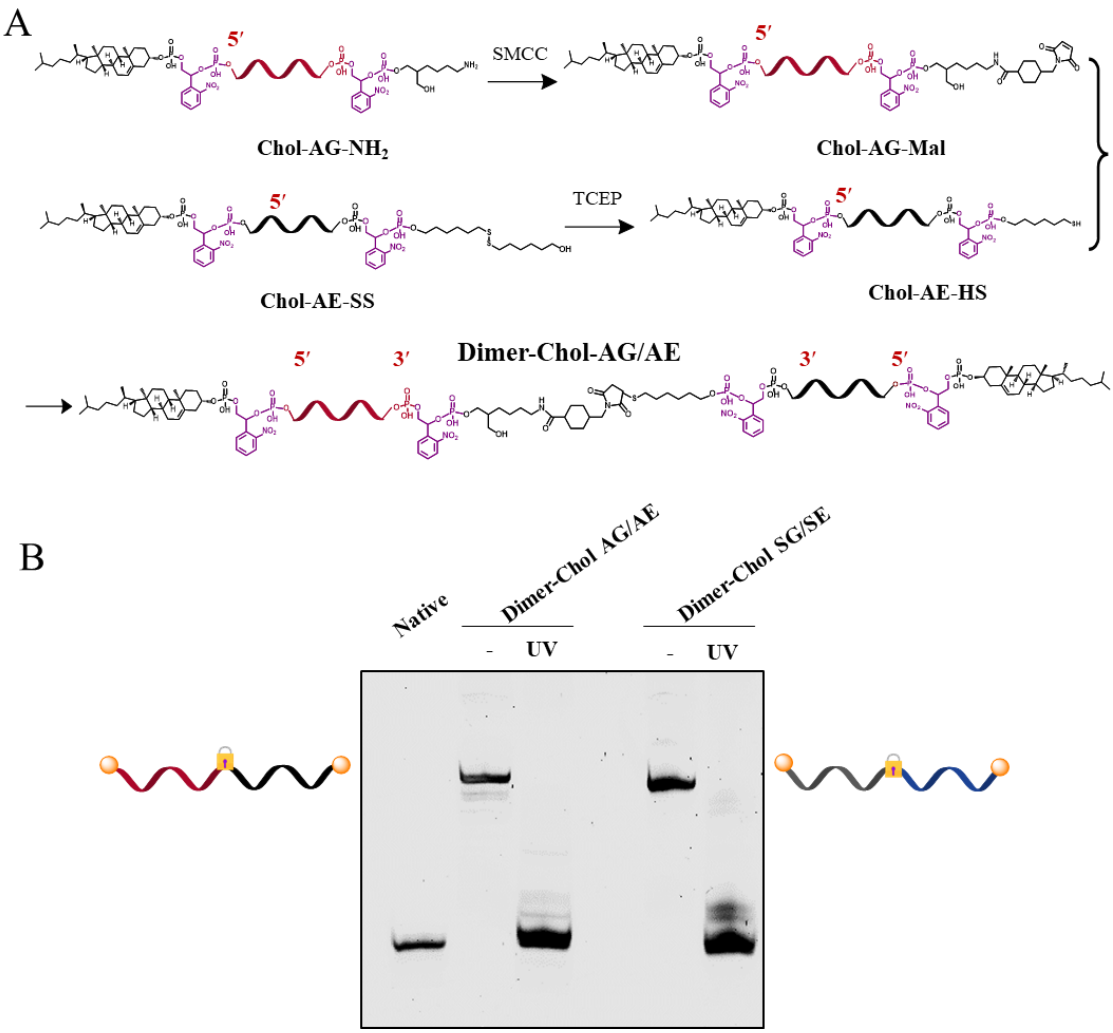


Figure S8. Synthesis of single-strand caged antisense Dimer-Chol-AG/AE (or caged sense Dimer-Chol-SG/SE) with 5′ end cholesterol modification (A). Dimerization and photorelease ability of caged Dimer-Chol-AG/AE and Dimer-Chol-SG/SE in PAGE gels (B).

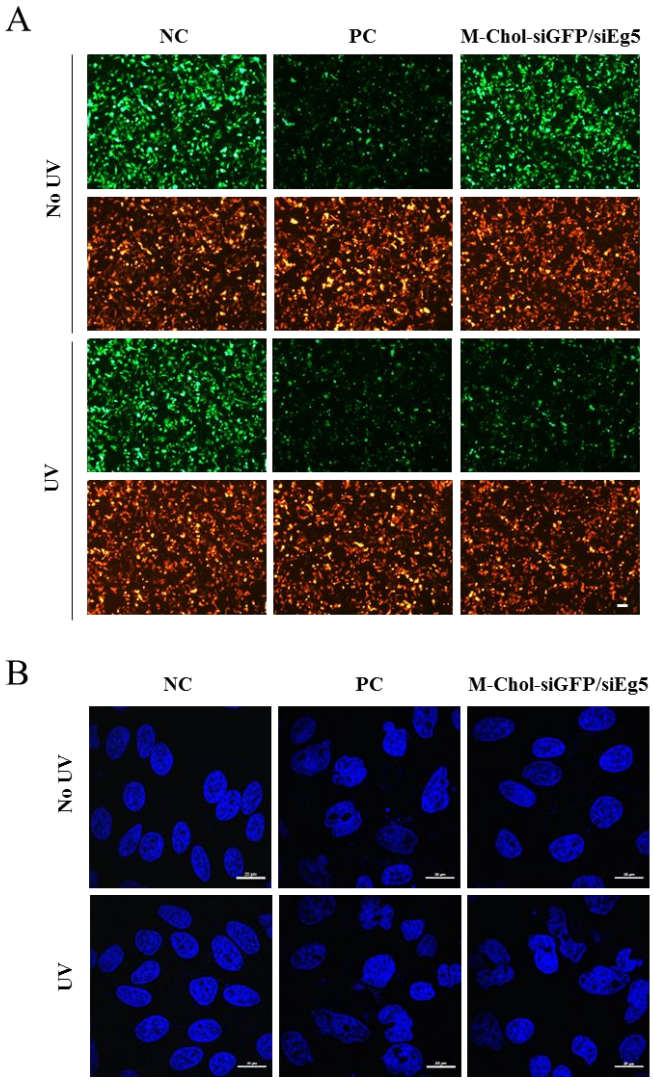


Figure S9. Photochemical regulation of GFP gene expression with *Two-in-One* caged Multi-Chol-siGFP/siEg5 self-assembled nanoparticles in HepG2 cells for 48 h incubation (A). Identifying nuclei phenotypes of HepG2 cells with *Two-in-One* caged Multi-Chol-siGFP/siEg5 nanoparticles using confocal laser scanning microscope (B).

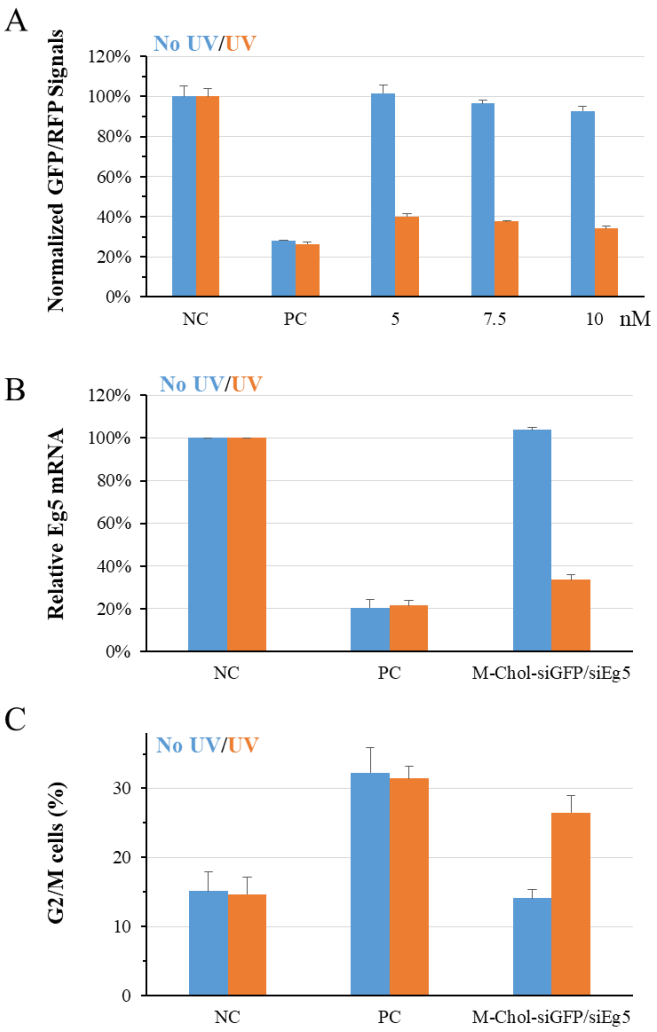
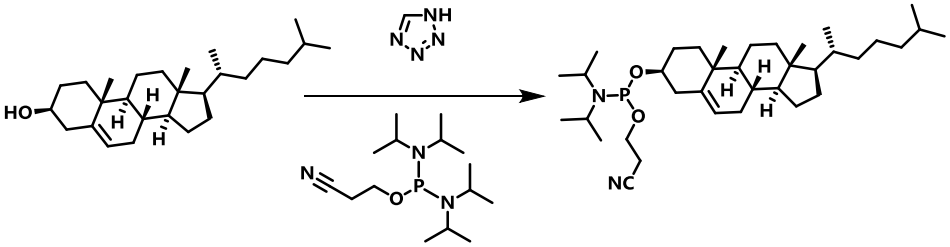
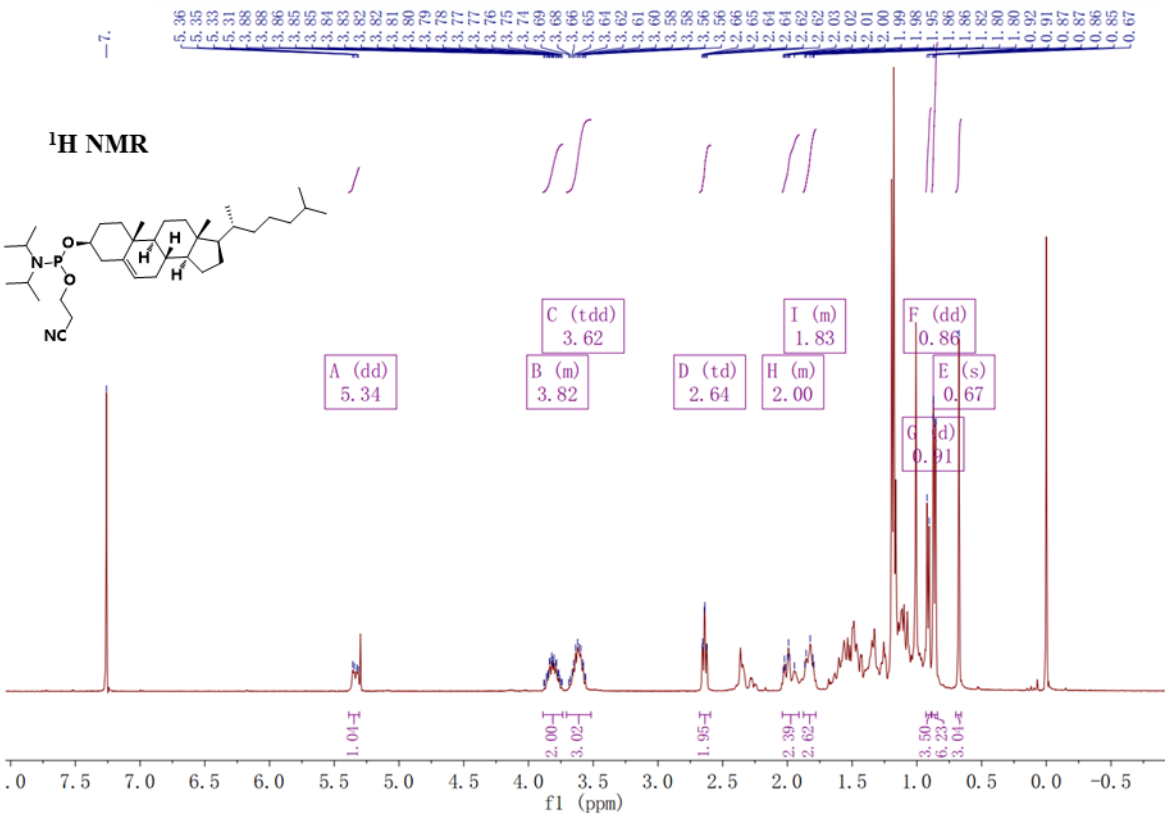


Figure S10. Simultaneous gene silencing activities of both GFP and Eg5 genes using *Two-in-One* caged Multi-Chol-siGFP/siEg5 self-assembled nanoparticles. (A) The photochemical regulation of GFP gene expression in HepG2 cells for 48 h incubation quantitated by flow cytometry. (B) Photochemical regulation of Eg5 mRNA levels determined by real time-qPCR using *Two-in-One* caged Multi-Chol-siGFP/siEg5 nanoparticles (7.5 nM siEg5 and 7.5 nM siGFP in nanoparticles). (C) Percentage of cells in G2/M phases of cell cycle. Data are presented as mean ± S.E.M. (n=3)

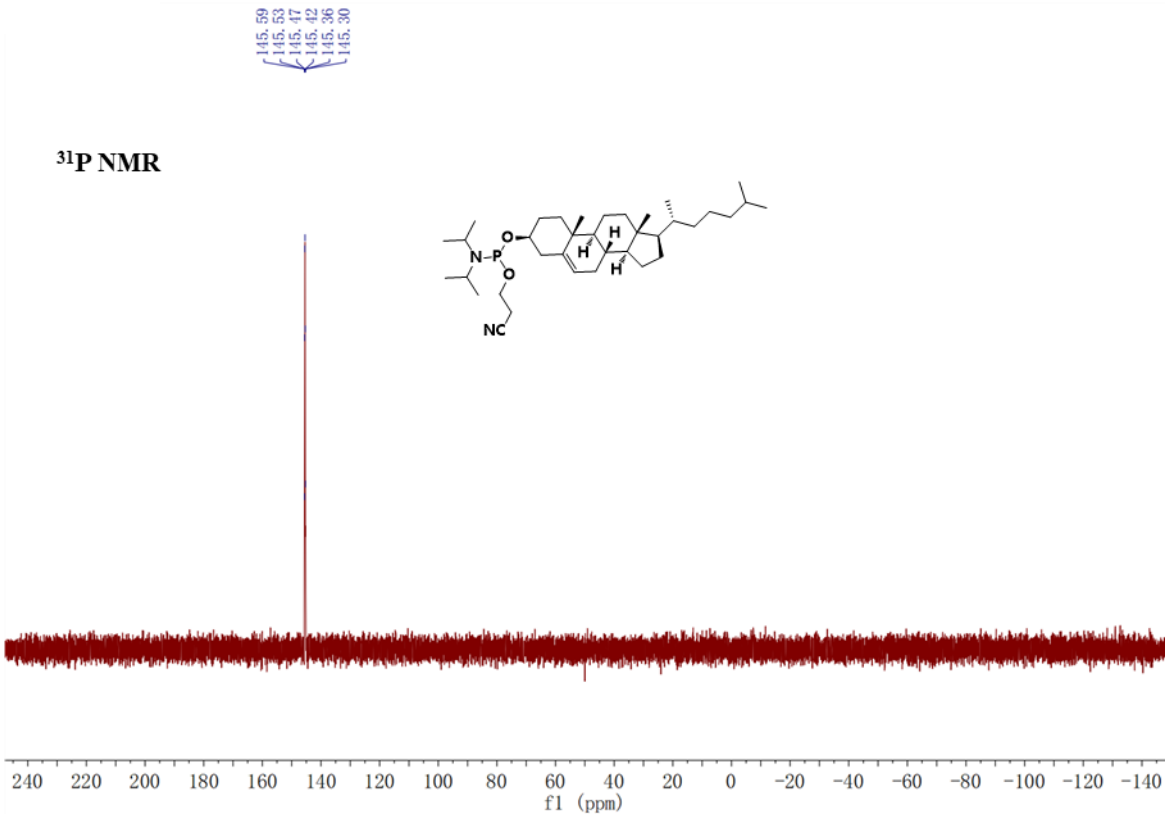
^{1}H NMR and ^{31}P NMR spectras of cholesterol phosphoramidite

The phosphoramidites of cholesterol (Chol) was synthesized according to our previous work confirmed by NMR.(1) ^{1}H NMR (400 MHz, Chloroform-d) δ 5.34 (dd, J = 12.7, 4.7 Hz, 1H), 3.89-3.74 (m, 2H), 3.62 (tdd, J = 13.7, 8.6, 5.6 Hz, 3H), 2.64 (td, J = 6.7, 1.8 Hz, 2H), 2.04-1.91 (m, 2H), 1.88-1.78 (m, 3H), 0.91 (d, J = 6.5 Hz, 4H), 0.86 (dd, J = 6.7, 1.7 Hz, 6H), 0.67 (s, 3H). ^{31}P NMR (162 MHz, CDCl3) δ 145.59, 145.53, 145.47, 145.42, 145.36, 145.30.

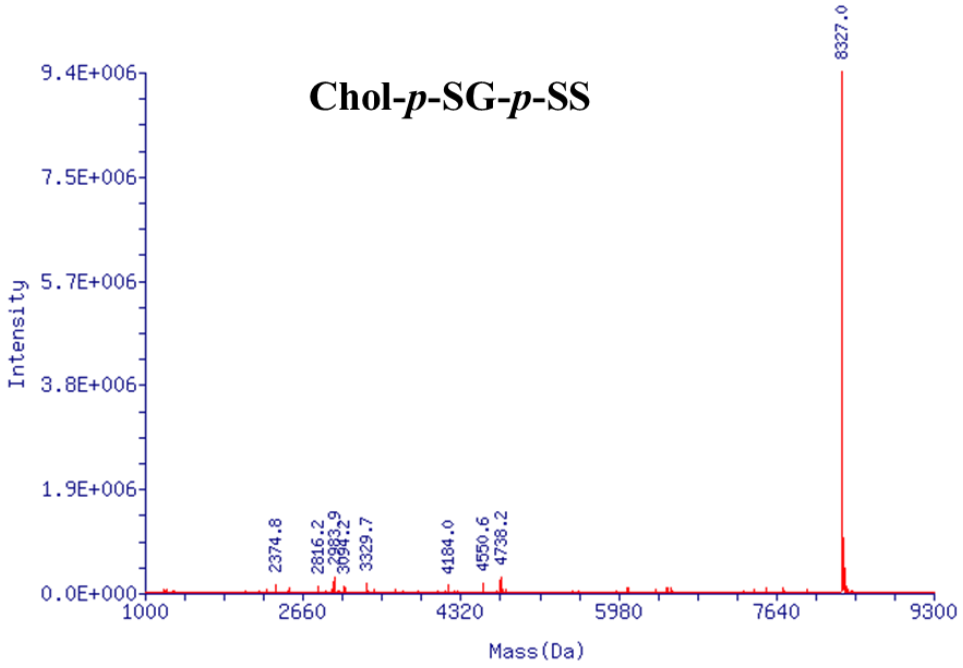


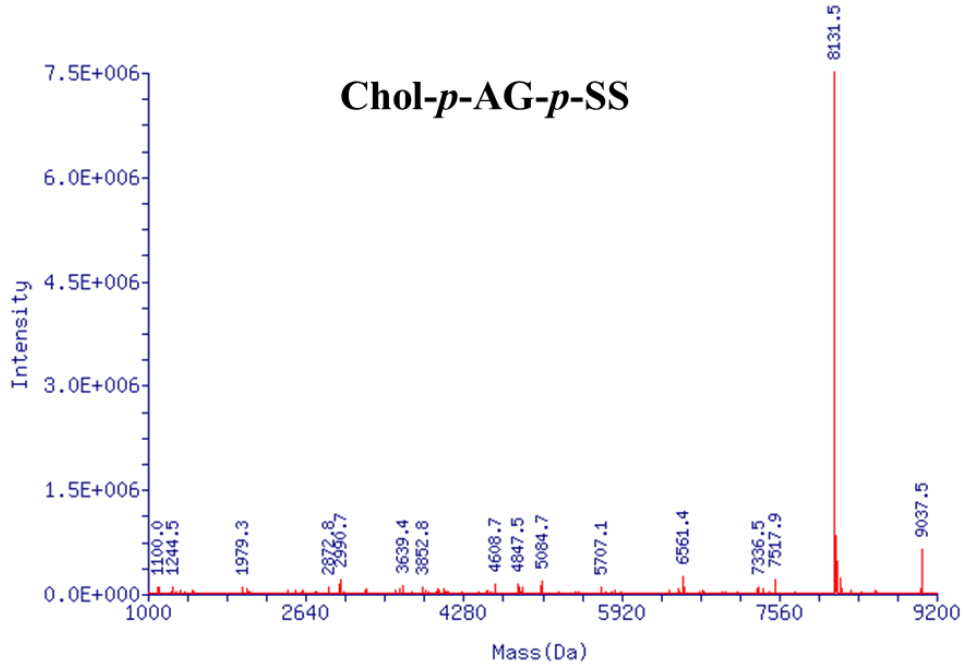


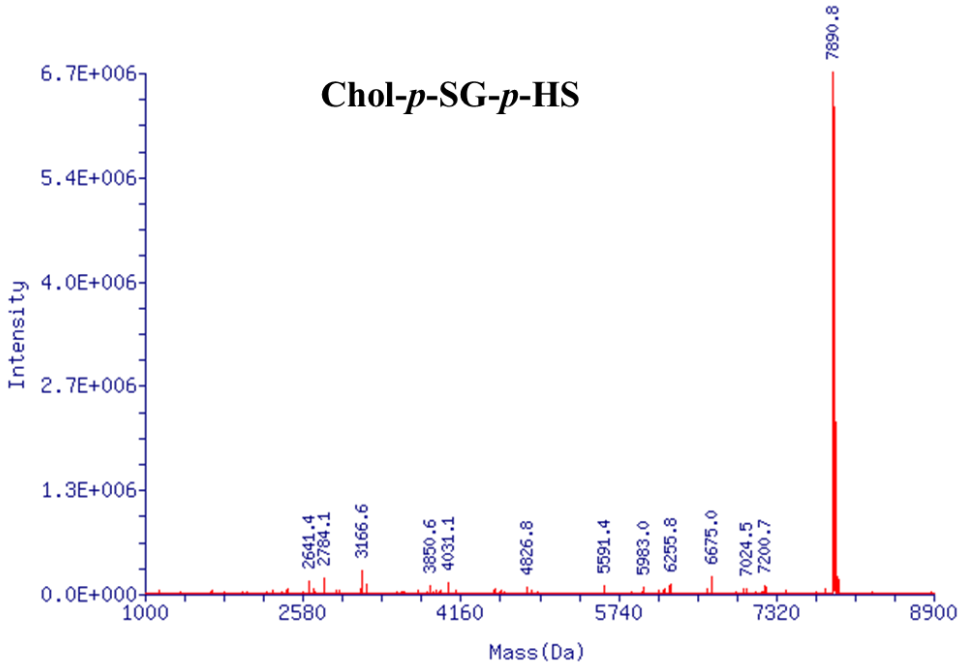
^{31}P NMR


145.59
145.53
145.47
145.42
145.36
145.30
O
N
P
O
NC
H
H
H
240 220 200 180 160 140 120 100 80 60 40 20 0 -20 -40 -60 -80 -100 -120 -140
f1 (ppm)

ESI-MS of chemically modified RNA oligonucleotides






Chol-p-SG-p-HS
Intensity
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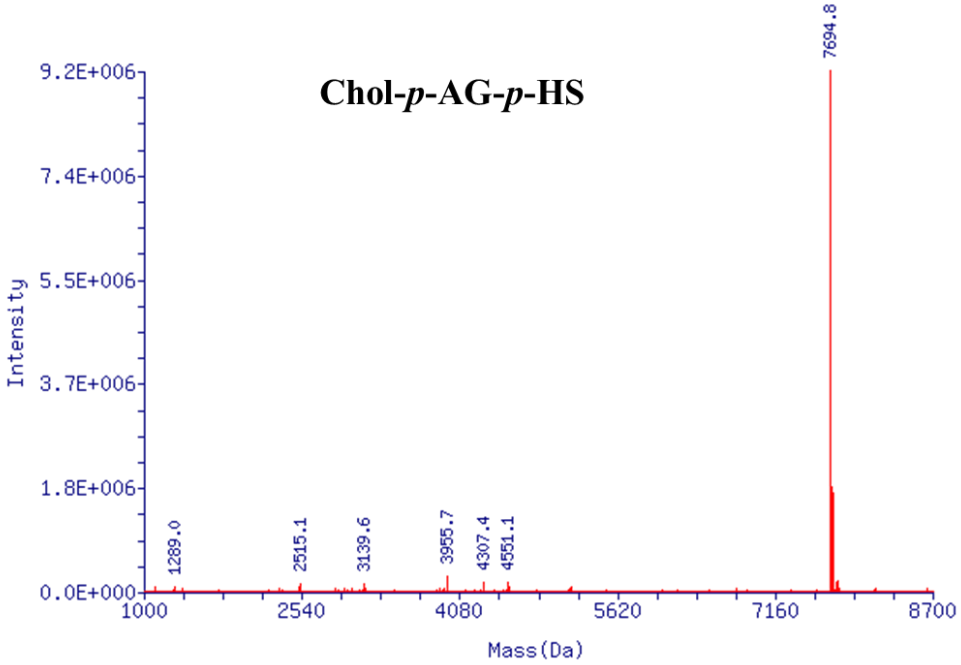
5.4E+006
4.0E+006
2.7E+006
1.3E+006
0.0E+000
1000
2580
4160
5740
7320
8900
Mass(Da)
7890.8
2641.4
2784.1
3166.6
3850.6
4031.1
4826.8
5591.4
5983.0
6255.8
6675.0
7024.5
7200.7


Chol-p-AG-p-HS
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7.4E+006
5.5E+006
3.7E+006
1.8E+006
0.0E+000
1000
2540
4080
5620
7160
8700
Mass(Da)
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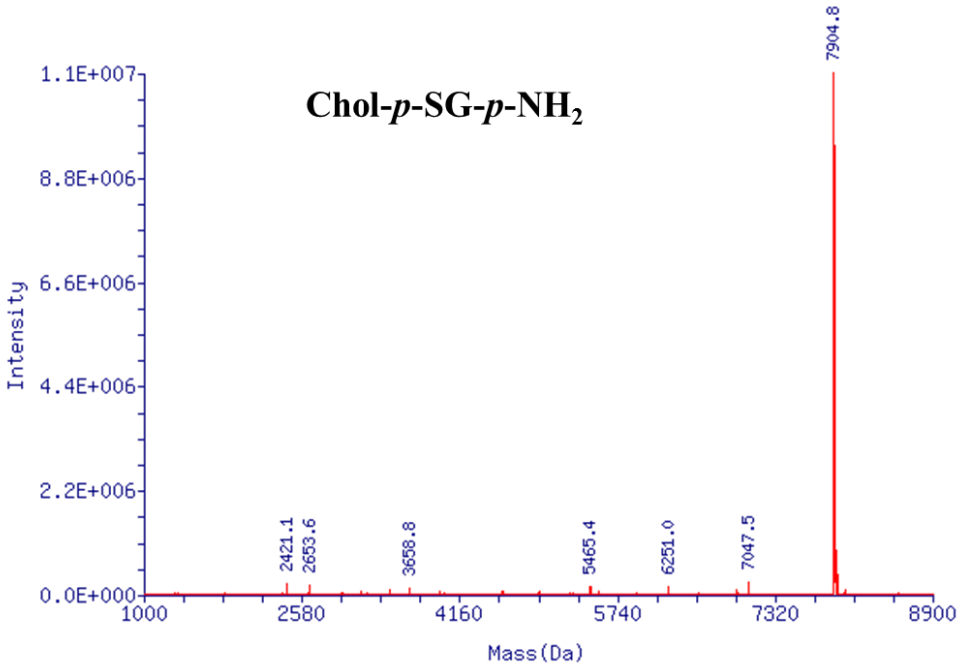
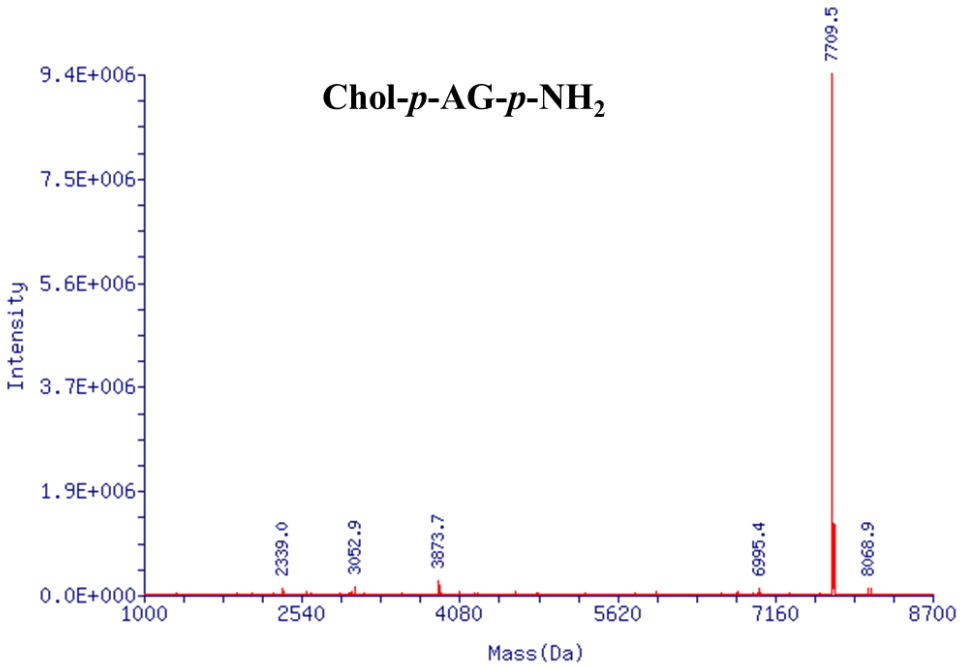
1289.0
2515.1
3139.6
3955.7
4307.4
4551.1


Chol-p-SG-p-NH2
1.1E+007
8.8E+006
6.6E+006
4.4E+006
2.2E+006
0.0E+000
Intensity
2421.1
2653.6
3658.8
5465.4
6251.0
7047.5
7904.8
1000
2580
4160
5740
7320
8900
Mass(Da)


Chol-p-AG-p-NH2
9.4E+006
7.5E+006
5.6E+006
3.7E+006
1.9E+006
0.0E+000
Intensity
2339.0
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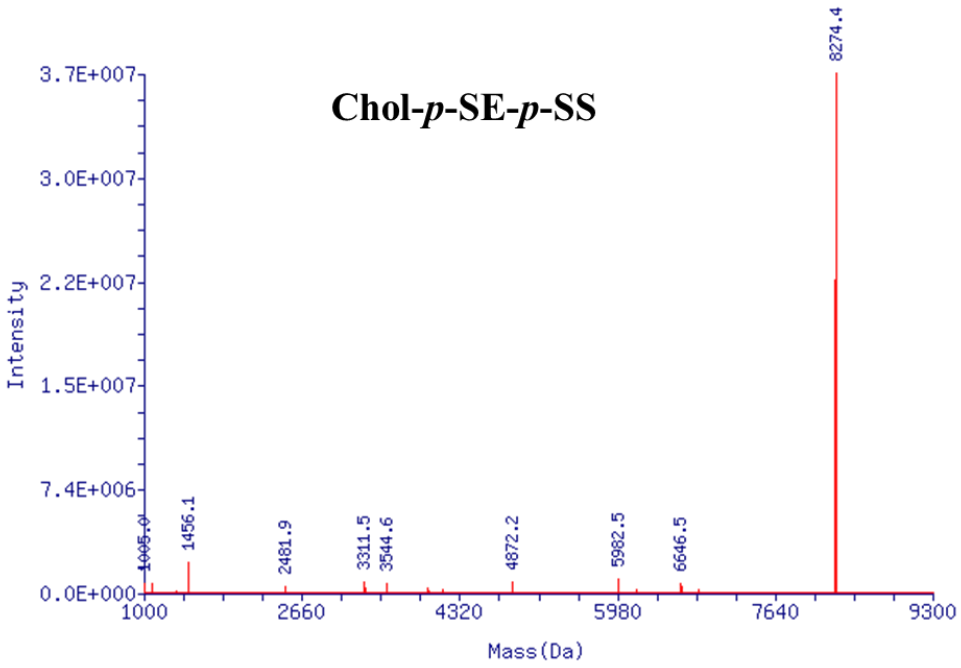
6995.4
7709.5
8068.9
1000
2540
4080
5620
7160
8700
Mass(Da)


Chol-*p*-SE-*p*-SS
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3.0E+007
2.2E+007
1.5E+007
7.4E+006
0.0E+000
Intensity
1000
2660
4320
5980
7640
9300
Mass(Da)
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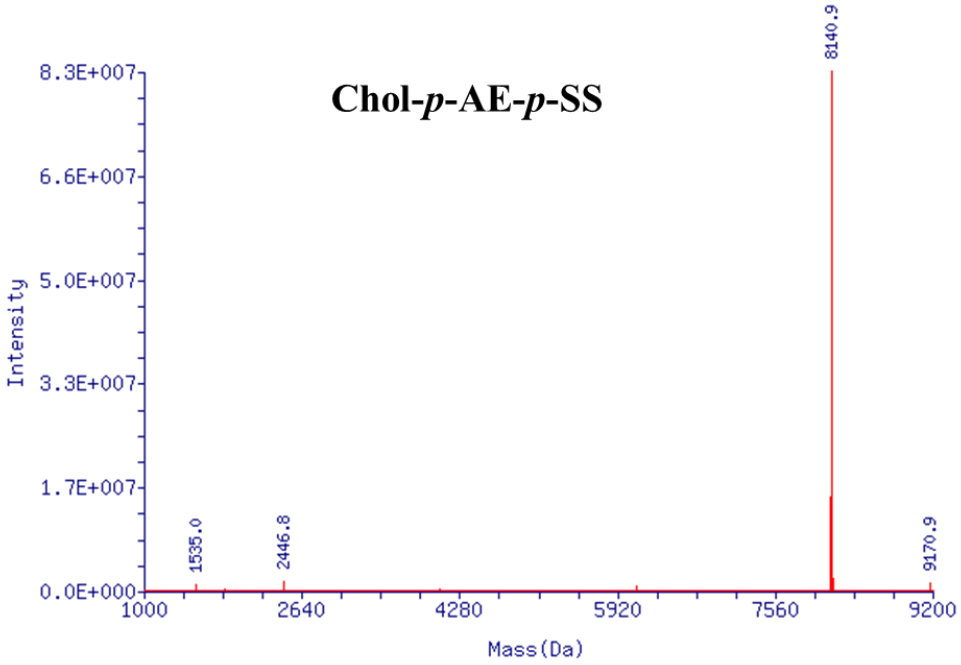
1456.1
2481.9
3311.5
3544.6
4872.2
5982.5
6646.5
8274.4


Chol-*p*-AE-*p*-SS
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6.6E+007
5.0E+007
3.3E+007
1.7E+007
0.0E+000
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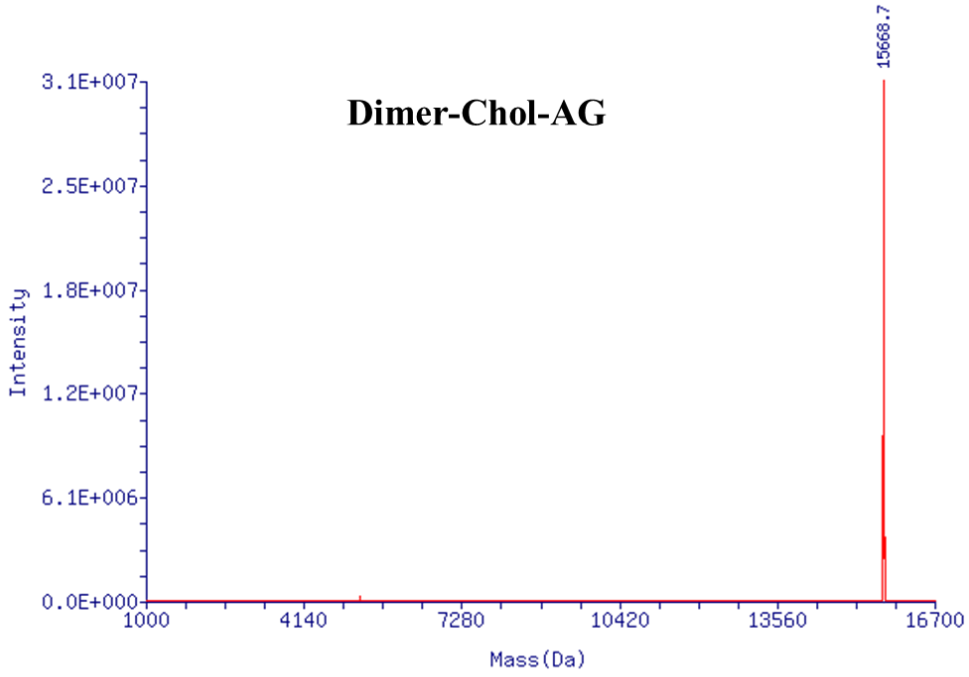
1000
2640
4280
5920
7560
9200
Mass(Da)
1535.0
2446.8
8140.9
9170.9


Dimer-Chol-AG
15668.7
3.1E+007
2.5E+007
1.8E+007
1.2E+007
6.1E+006
0.0E+000
Intensity
1000
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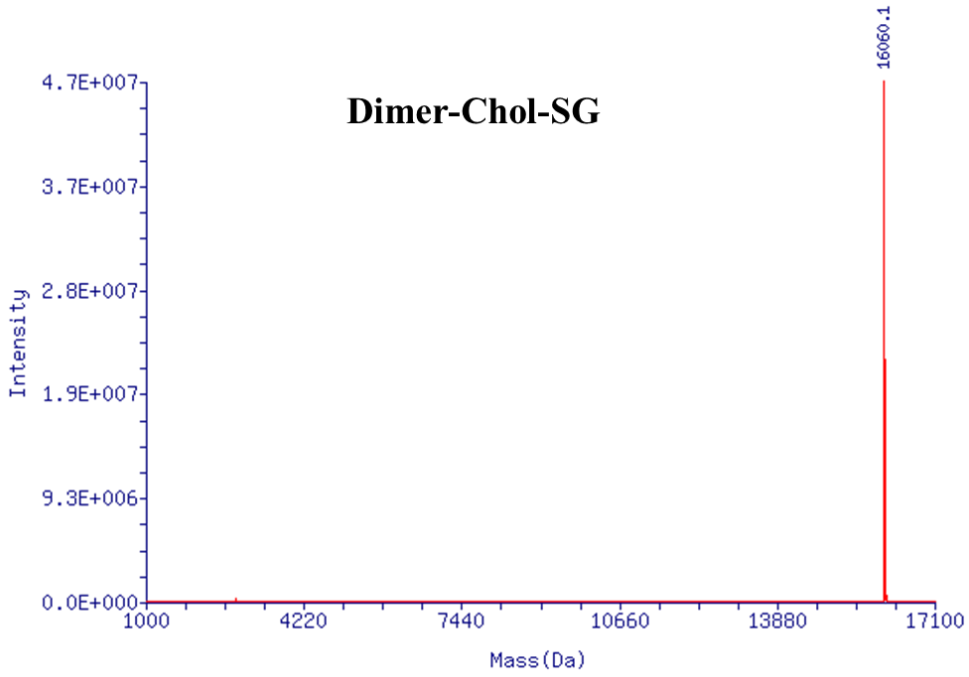
7280
10420
13560
16700
Mass(Da)


Dimer-Chol-SG
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3.7E+007
2.8E+007
1.9E+007
9.3E+006
0.0E+000
Intensity
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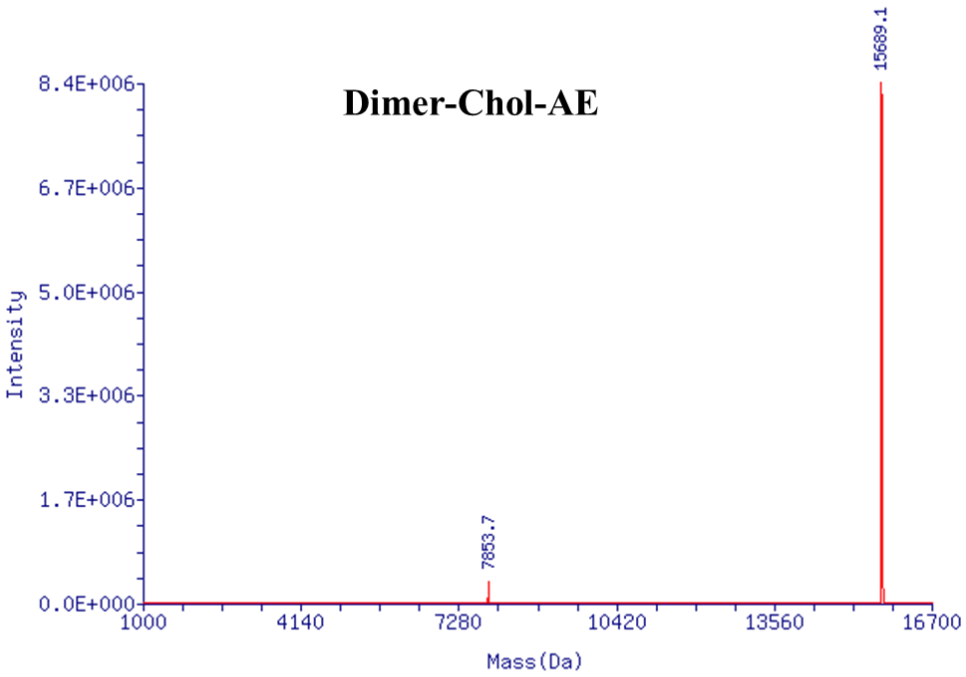
10660
13880
17100
Mass(Da)


Dimer-Chol-AE
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7853.7
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6.7E+006
5.0E+006
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Intensity
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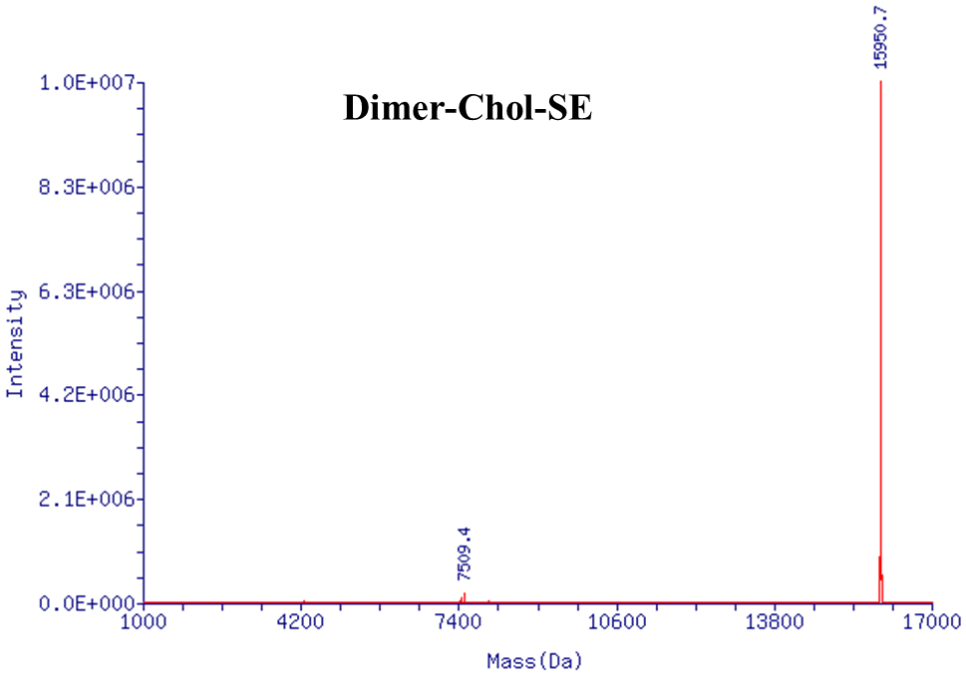
4140
7280
10420
13560
16700
Mass(Da)


Dimer-Chol-SE
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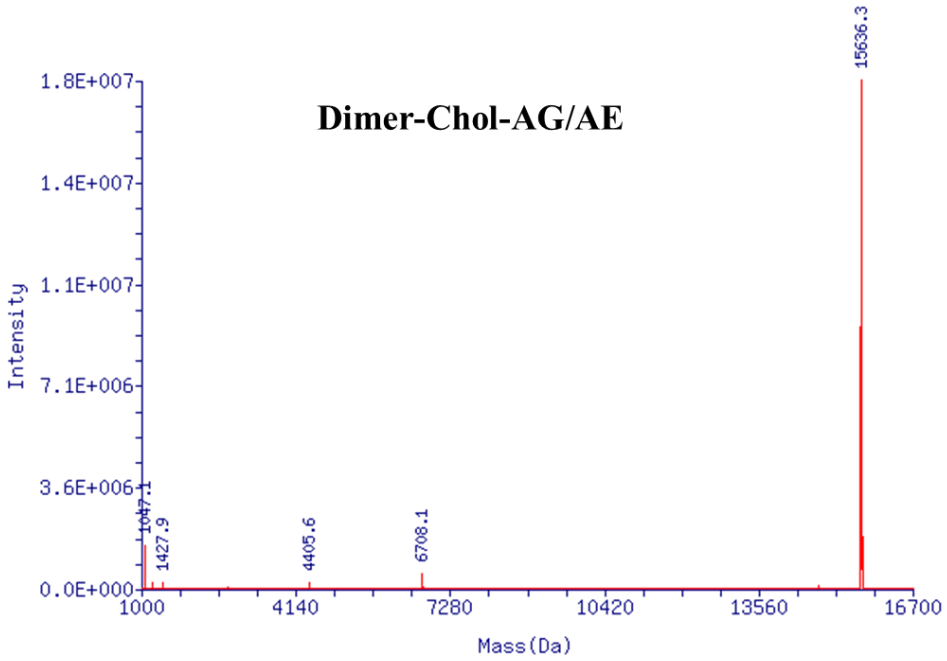
7509.4
1.0E+007
8.3E+006
6.3E+006
4.2E+006
2.1E+006
0.0E+000
Intensity
1000
4200
7400
10600
13800
17000
Mass(Da)


Dimer-Chol-AG/AE
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1.4E+007
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3.6E+006
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4405.6
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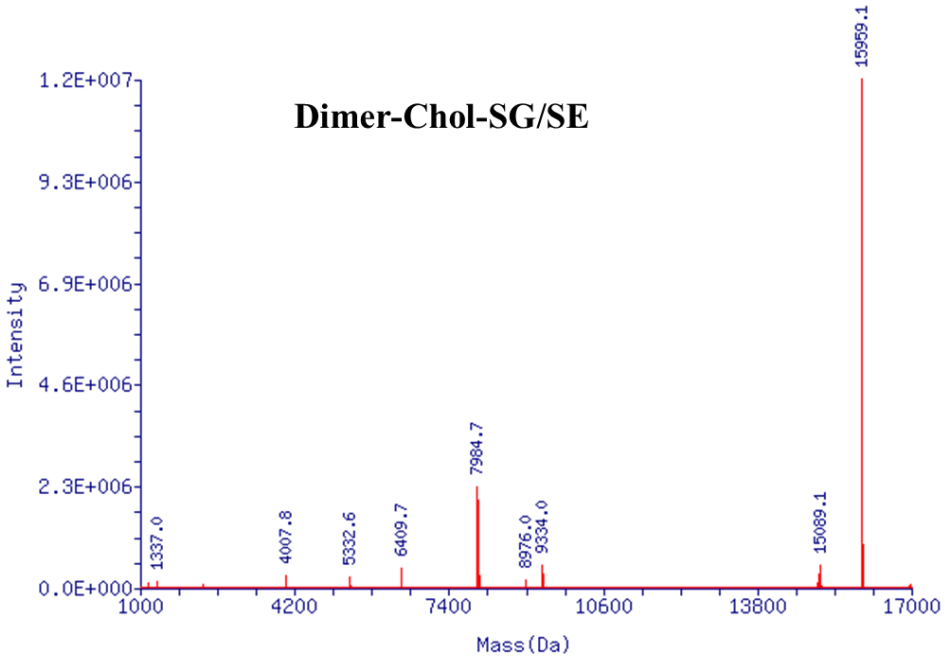
15636.3
1000
4140
7280
10420
13560
16700
Mass(Da)


Dimer-Chol-SG/SE
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5332.6
6409.7
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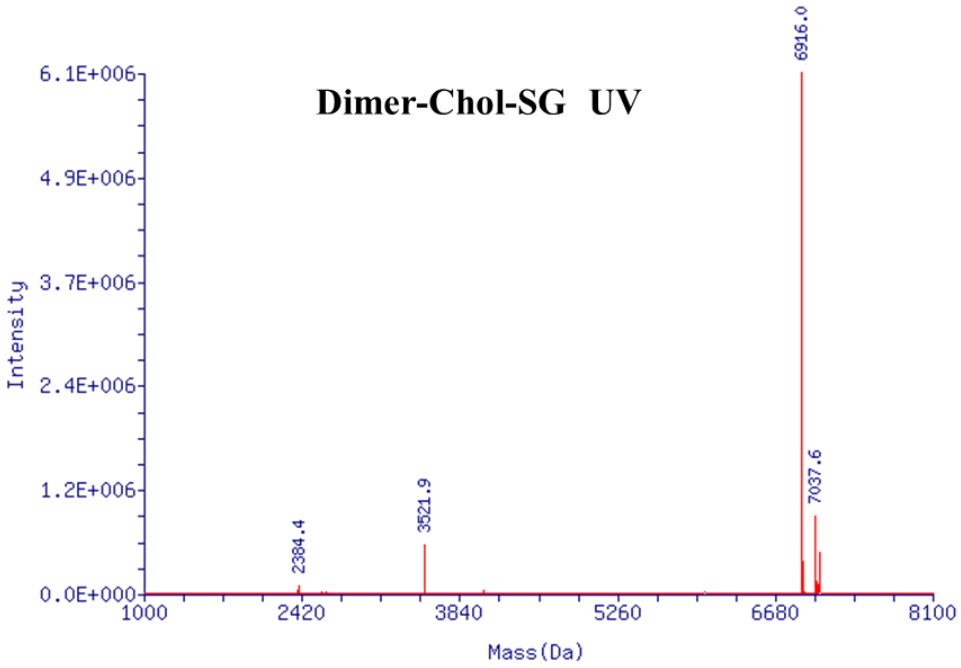
4200
7400
10600
13800
17000
Mass(Da)



REFERENCE

1. Yang, J., Chen, C. and Tang, X. (2018) Cholesterol-Modified Caged siRNAs for Photoregulating Exogenous and Endogenous Gene Expression. *Bioconjug Chem*, **29**, 1010-1015.