Supplementary information

Dextran-Conjugated Caged siRNA Nanoparticles for Photochemical Regulation of RNAi-Induced Gene Silencing in Cells and Mice

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

Target	Name	Sequence (5')
GFP	SG	GAA CGG CAU CAA GGU GAA CTT
	AG	GUU CAC CUU GAU GCC GUU CTT
	SS-*p*-SG	SS-PL-GAA CGG CAU CAA GGU GAA CTT
	SS-*p*-AG	SS-PL-GUU CAC CUU GAU GCC GUU CTT
Eg5	SE	CAA CAA GGA UGA AGU CUA UTT
	AE	AUA GAC UUC AUC CUU GUU GTT
	SS-*p*-SE	SS-PL-CAA CAA GGA UGA AGU CUA UTT
	SS-*p*-AE	SS-PL-AUA GAC UUC AUC CUU GUU GTT

S, sense RNA strand; A, antisense RNA strand; G, GFP; E, Eg5; SS, 5′-thiol modifier C6 S-S; *p* or PL, photolinker

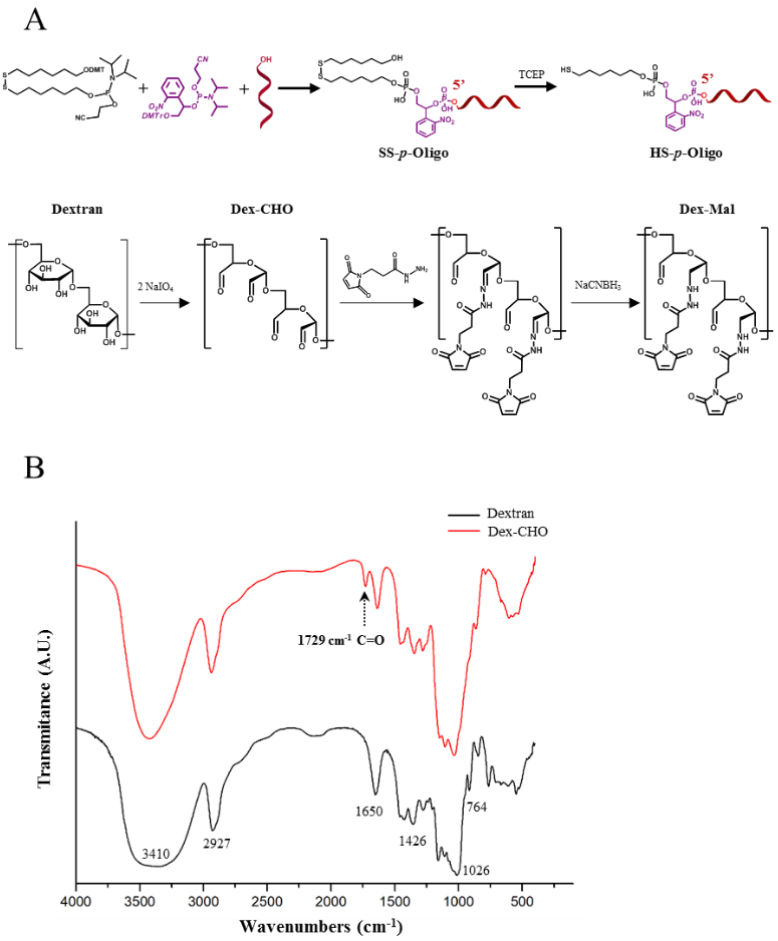


Figure S1. Synthesis of thiol-modified caged siRNAs and maleimide-functionalized of dextran (A) and analysis of the aldehyde groups of dextran (Dex-CHO) by FTIR (B).

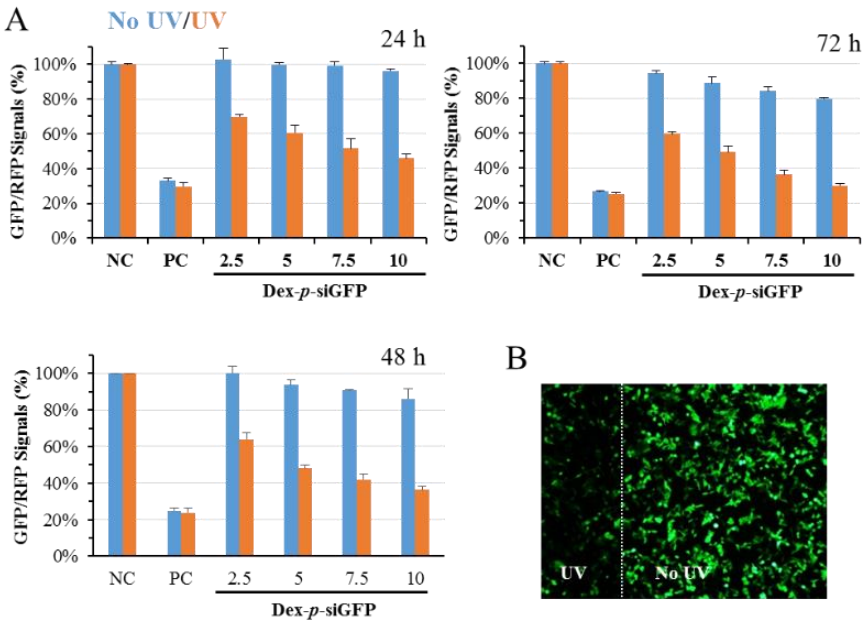


Figure S2. Photoregulation of GFP gene silencing using the caged Dex-*p*-siGFP nanoparticles. Time- and dose-dependent photomodulation GFP expression quantified using flow cytometry (A). Patterning experiments revealed that Dex-*p*-siRNA could spatial control of gene expression via simple light irradiation(B). PC, positive control siGFP (AG/SG)

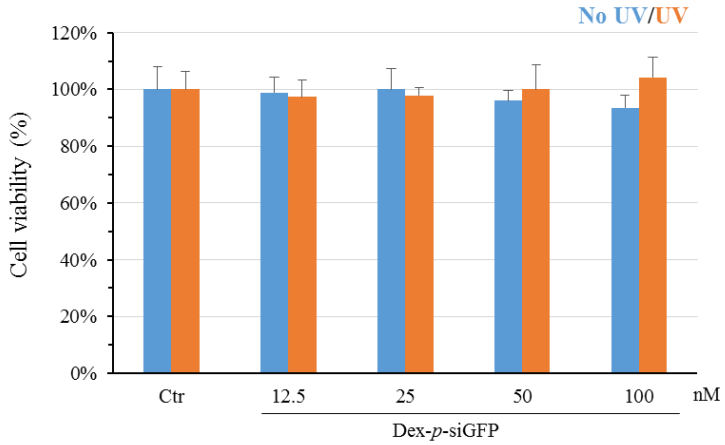


Figure S3. The effect of dextran-conjugated caged siGFP on cell proliferation for 48 h at the concentration of 12.5-100 nM.

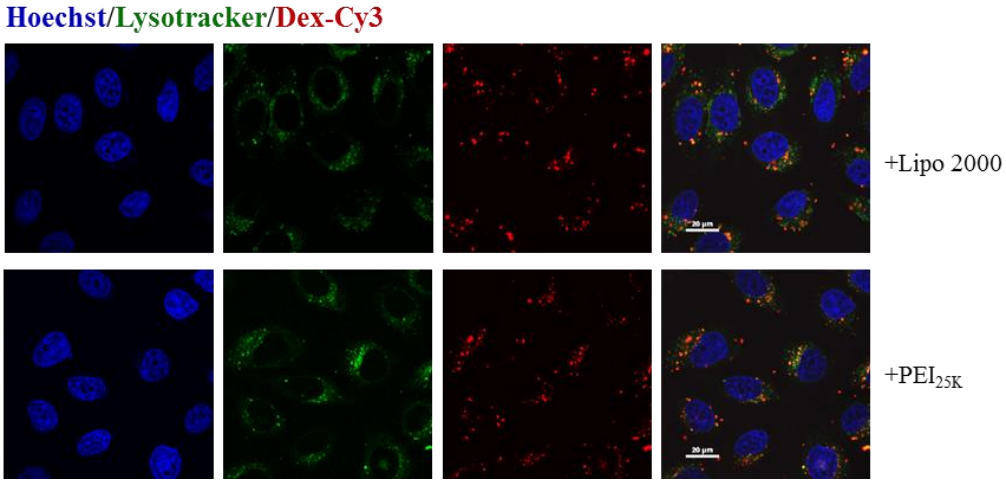


Figure S4. Subcellular localization of Cy3-labeled dextran-conjugated caged siGFP (Dex-Cy3). The cells were stained with Lysotracker green and Hoechst 33342 after 5 h transfection with Lipo 2000 or PEI $_{25K}$ agent.

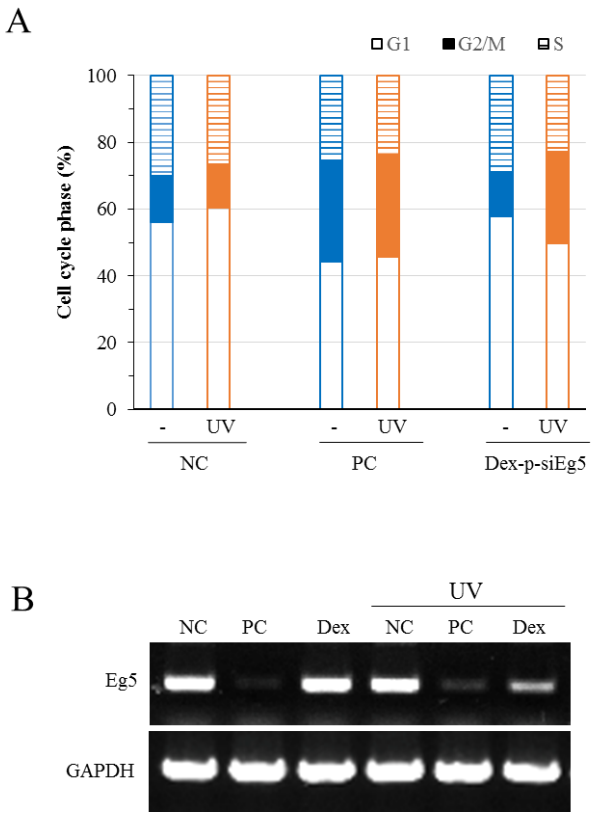


Figure S5. Photochemical regulation of Eg5 gene expression with the caged Dex-*p*-siEg5 nanoparticles. Cell cycle perturbation and analysis of mitotic arrest stained with propidium iodide (A). Photochemical regulation of Eg5 gene expression by RT-PCR (B). PC, positive control siEg5 (AE/SE); NC, negative control.

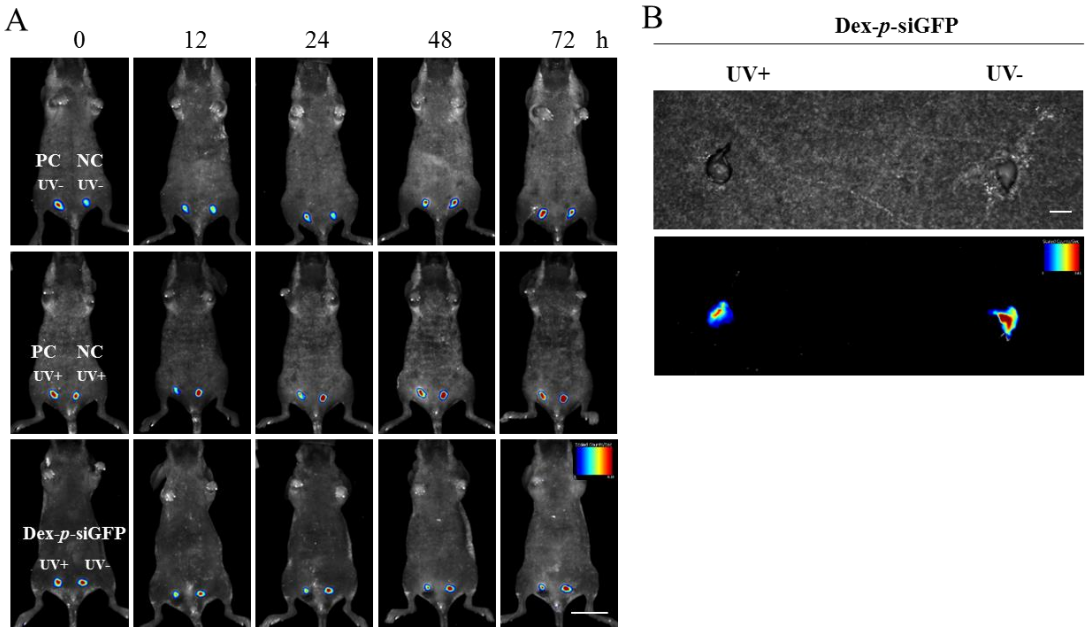


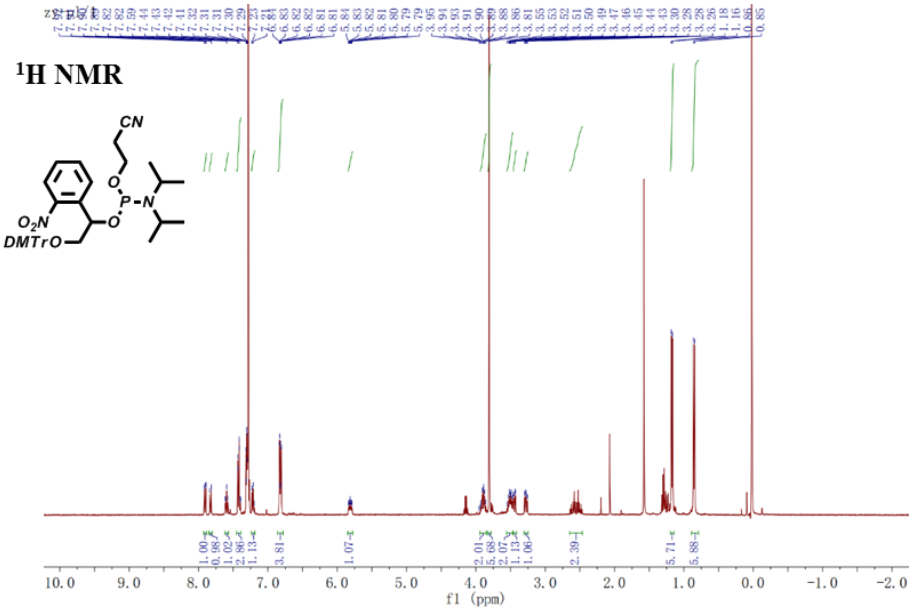
Figure S6. Photochemical regulation of GFP gene expression *in vivo* with the caged Dex-*p*-siGFP nanoparticles. *In vivo* real-time fluorescence images of tumor-bearing mice at different time points after intratumoral administration of 3 nmol of the native siRNA or caged Dex-*p*-siRNA nanoparticles. Scale bar = 1.5 cm. (A). Representative images of sectioned tumors injected with caged Dex-*p*-siRNA nanoparticles and left tumor was subjected to light irradiation (UV+). Bright field image (Up) and Overlapped image (down) (B). PC, positive control siGFP (AG/SG); NC, negative control, PBS buffer injection. Scale bar = 4 mm.

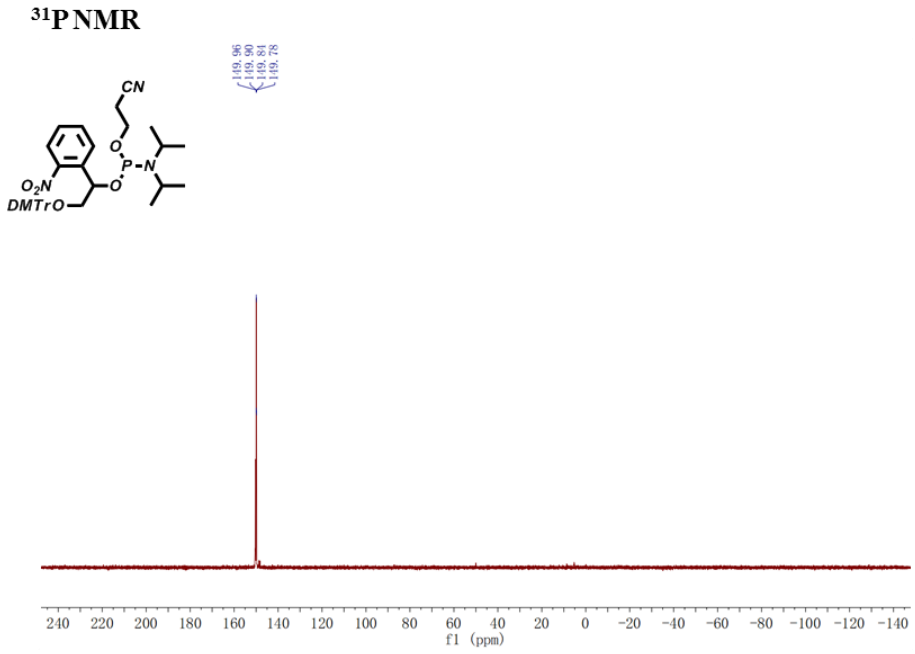
Table S2. The fluorescence intensity of tumor was quantified at different time points

Time	NC UV-	NC UV+	PC UV-	PC UV+	Dex UV-	Dex UV+
0 h	7536666.6± 355574	12686667± 4588740	16733333± 1650253	21200000± 1272792	12800000± 3026549	17566667± 3111270
12 h	7790000± 427200	13866667± 4219400	6000000± 3613475	5573333± 2673431	11053333± 3022339	5750000± 1414214
24 h	7873333.3± 816843	15733333± 5896892	5956667± 4422401	7910000± 3496613	14370000± 4989760	7910000± 4313351
48 h	11113333.3± 2358502	18900000± 3459769	7949000± 5805894	12410000± 4261842	15403333± 4545881	8343333± 3323402
72 h	10566666.6± 472581	18533333± 4384442	13076667± 6742895	16966667± 8197764	15733333± 3000556	11396667± 2757716

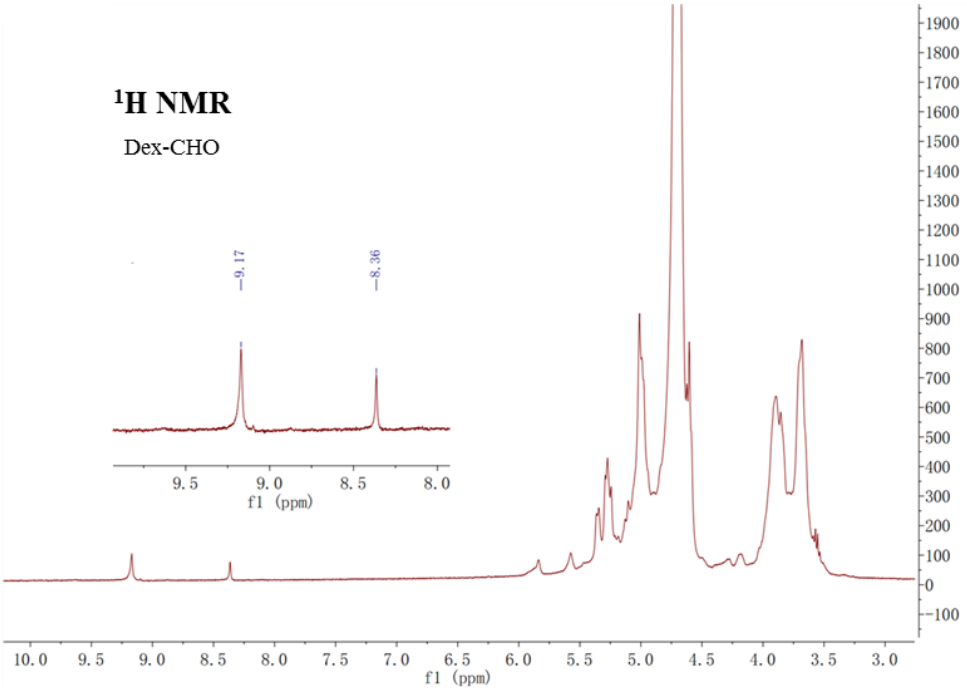
^{1}H NMR and ^{31}P NMR spectras of photolinker phosphoramidite

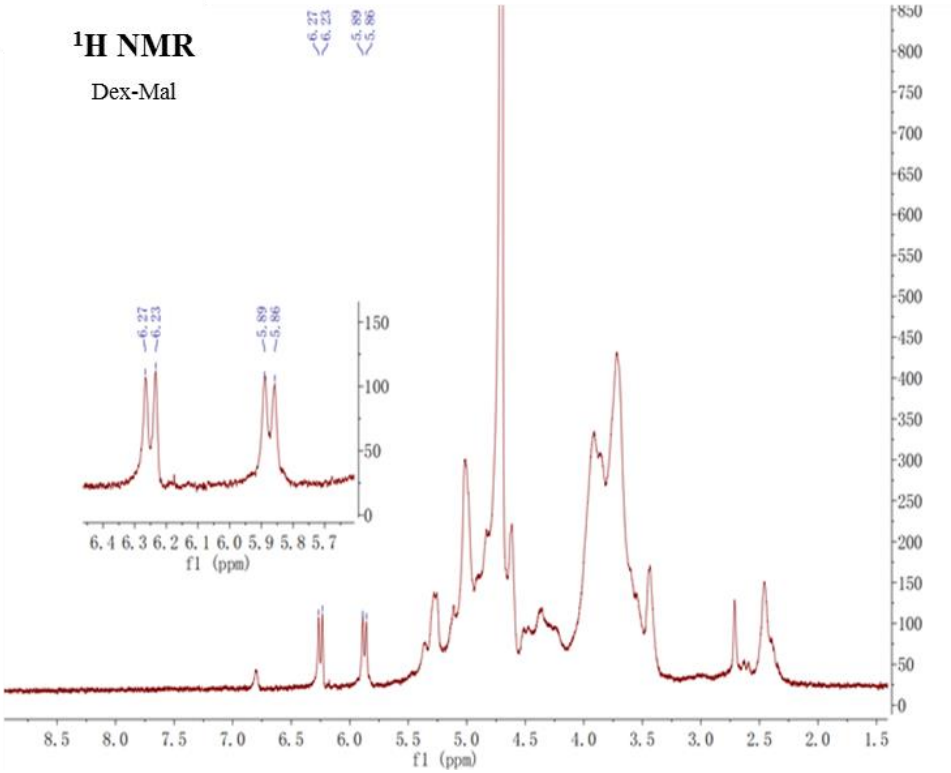
The phosphoramidite of photolablie linker (PL or p) was synthesized according to standard phosphoramidite synthetic protocol[1, 2]. ^{1}H NMR(400 MHz) and ^{31}P NMR(162 MHz) spectras were taken on Bruker AVANCE III-400 spectrometers and standardized to the NMR solvent peak, chemical shifts were reported in parts per million (ppm). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (dd, J = 8.2, 1.3 Hz, 1H), 7.85 – 7.81 (m, 1H), 7.59 (t, J = 7.6 Hz, 1H), 7.42 (td, J = 8.0, 7.5, 1.5 Hz, 3H), 7.33 – 7.29 (m, 3H), 7.21 (t, J = 7.2 Hz, 1H), 6.82 (dd, J = 9.0, 2.8 Hz, 4H), 5.81 (ddd, J = 10.6, 6.8, 3.2 Hz, 1H), 3.94 – 3.85 (m, 2H), 3.81 (s, 6H), 3.50 (dt, J = 10.4, 6.8 Hz, 1H), 3.45 (dd, J = 9.5, 3.2 Hz, 1H), 3.28 (dd, J = 9.5, 6.9 Hz, 1H), 2.65 – 2.46 (m, 2H), 1.17 (d, J = 6.8 Hz, 6H), 0.85 (d, J = 6.8 Hz, 6H). ^{31}P NMR (162 MHz, $CDCl_3$) δ 149.85 (d, J = 9.6 Hz).





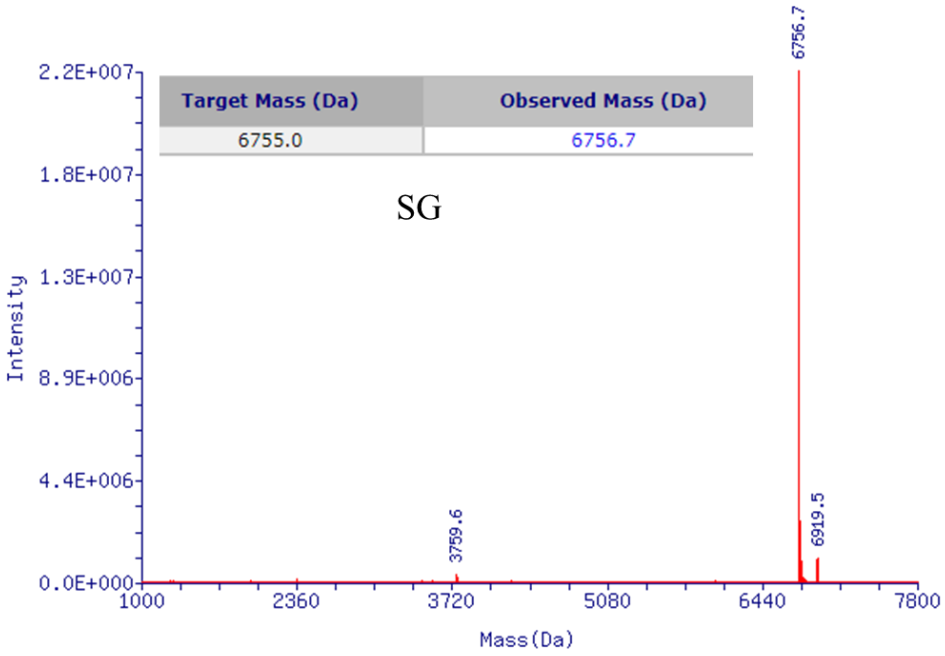
The partial oxidation of dextran (Dex-CHO) and maleimide-functionalized of dextran (Dex-Mal) were prepared according to the previous reported procedures[3]. The aldehyde groups of dextran (Dex-CHO) ^{1}H NMR (400 MHz, D_2O) δ 9.17 (s, 1H), δ 8.36 (s, 1H). The maleimide groups of dextran (Dex-Mal) ^{1}H NMR (400 MHz, Deuterium Oxide) δ 6.25 (d, J = 13.2 Hz, 1H), 5.87 (d, J = 12.9 Hz, 1H).

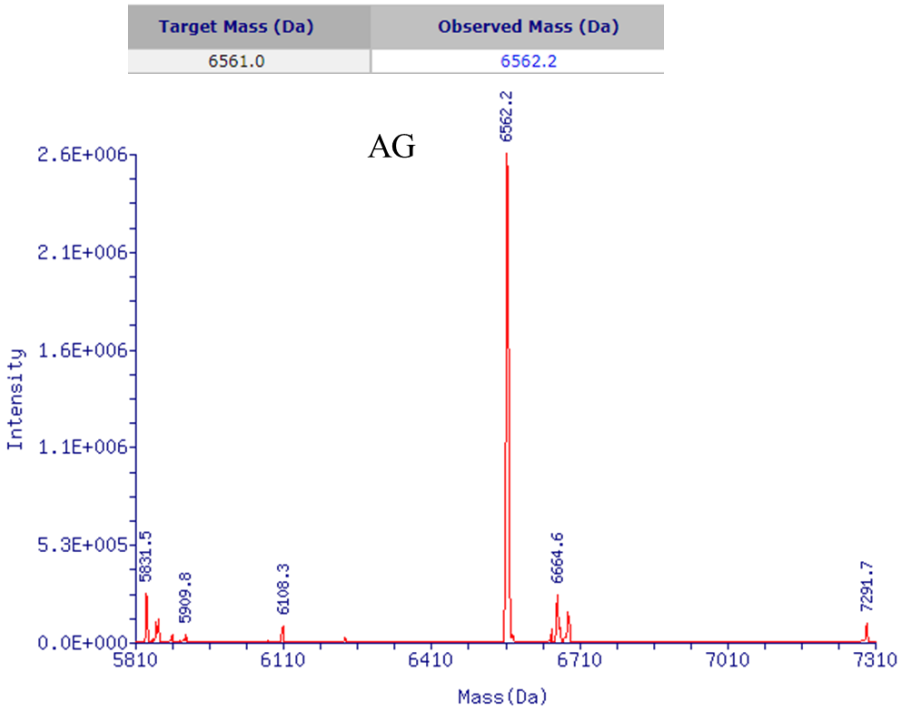




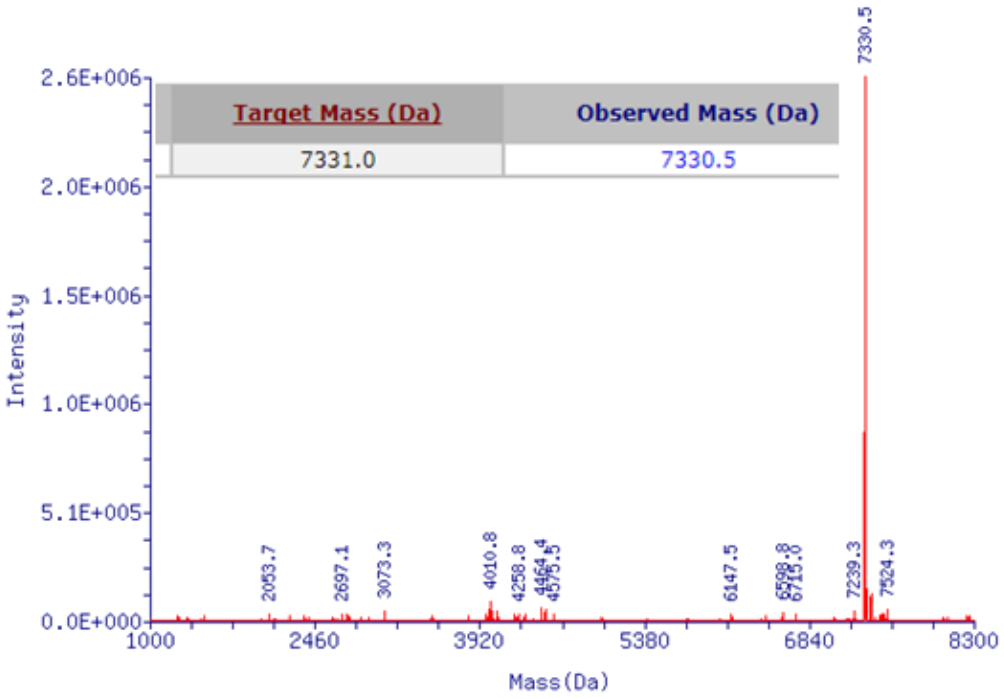
ESI-MS of native or thiol-modifier RNA oligonucleotides

These native or 5’ thiol-modified caged RNA oligonucleotides targeting GFP and Eg5 (SS-*p*-oligos) were further purified using reversed-phase HPLC and characterized by ESI-MS. The sequences of caged thiol-modified oligonucleotides used in this study.





SS-*p*-SG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Observed Mass (Da)
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7330.5
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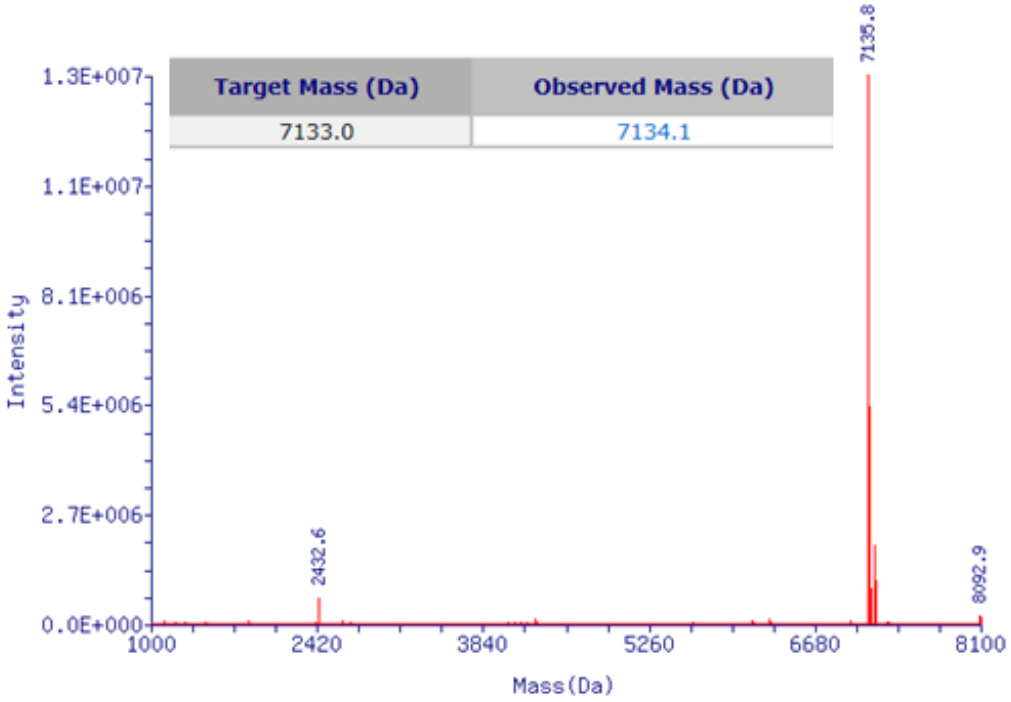
2.0E+006
1.5E+006
1.0E+006
5.1E+005
0.0E+000
1000
2460
3920
5380
6840
8300
Mass(Da)
7330.5
2053.7
2697.1
3073.3
4010.8
4258.8
4464.4
4575.5
6147.5
6598.8
6715.0
7239.3
7524.3

SS-*p*-AG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Observed Mass (Da)
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7134.1
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8.1E+006
5.4E+006
2.7E+006
0.0E+000
1000
2420
3840
5260
6680
8100
Mass(Da)
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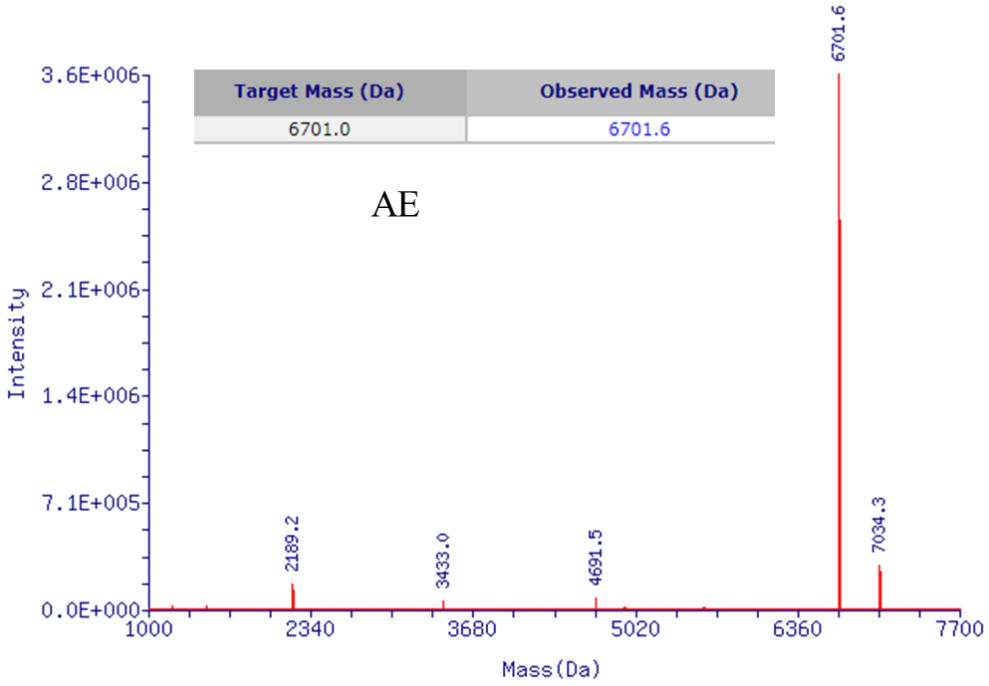
2432.6
8092.9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Observed Mass (Da)
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7.1E+005
0.0E+000
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4691.5
6701.6
7034.3
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2340
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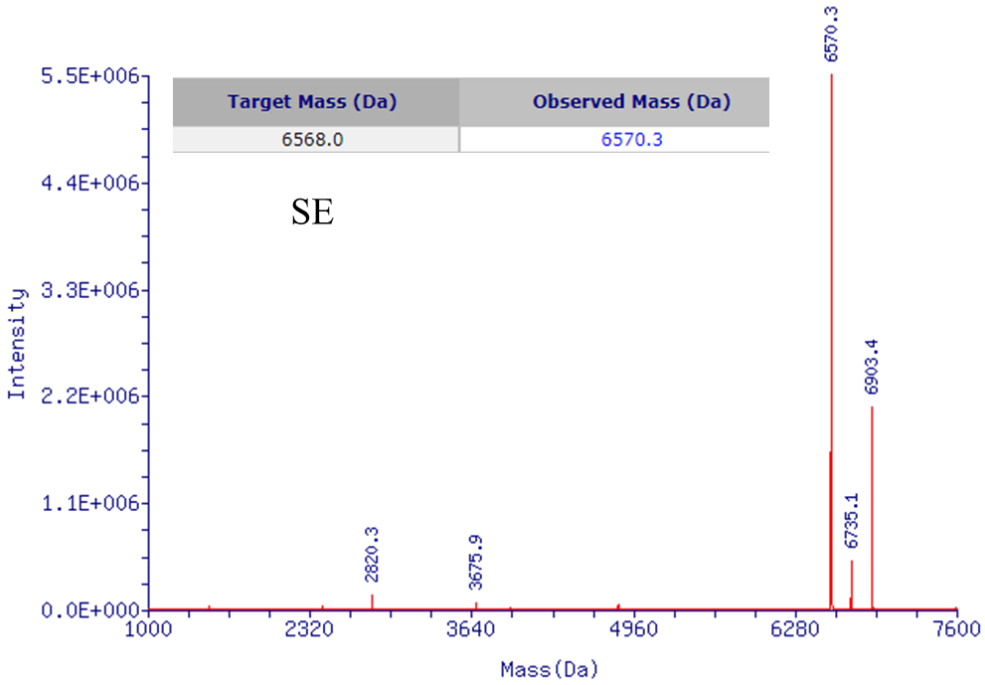
5020
6360
7700
Mass(Da)


Target Mass (Da)
Observed Mass (Da)
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6570.3
SE
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3.3E+006
2.2E+006
1.1E+006
0.0E+000
2820.3
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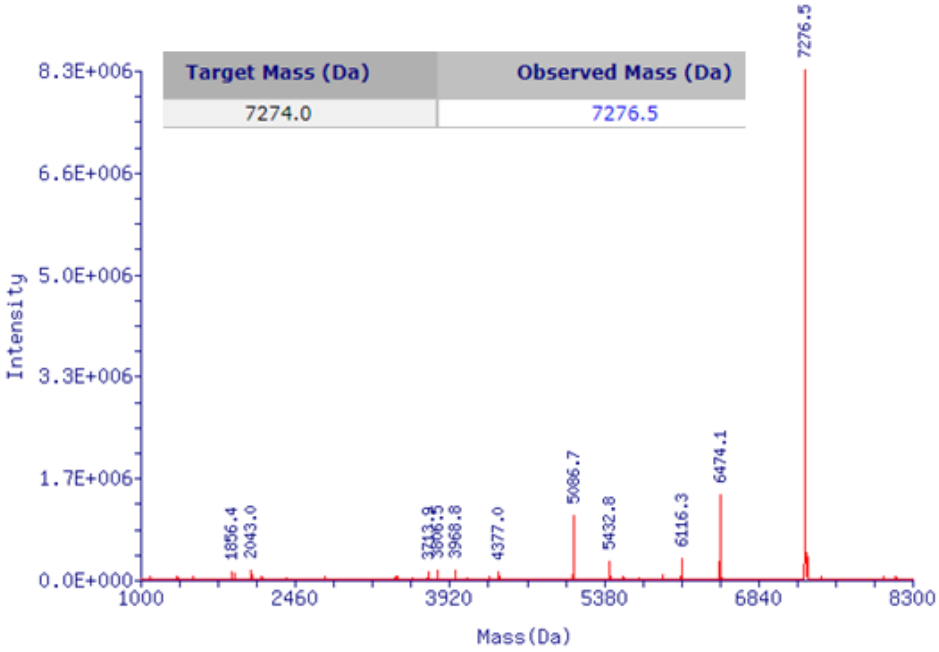
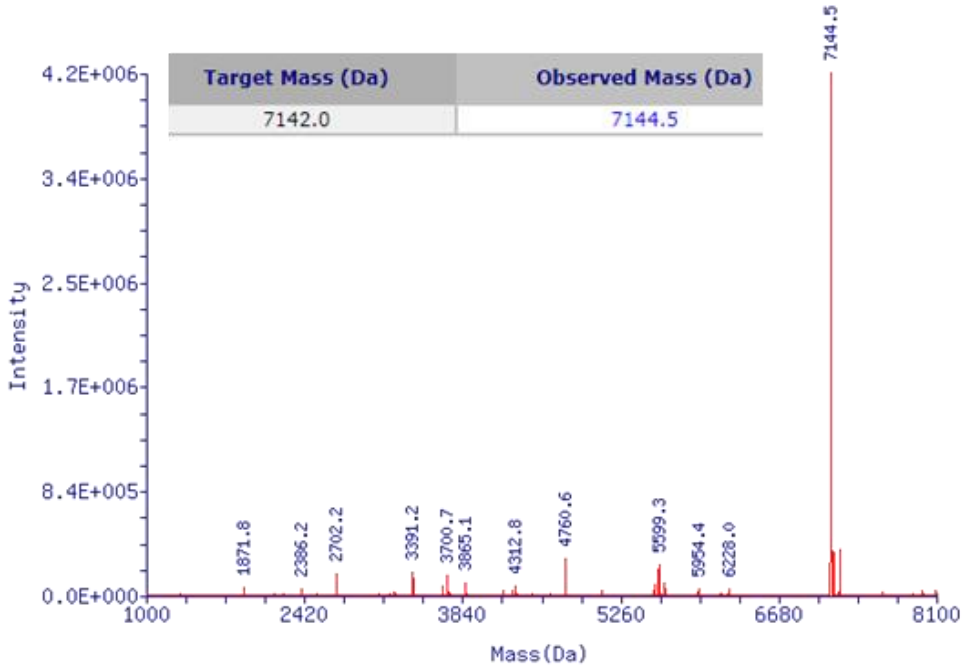
6735.1
6903.4
1000
2320
3640
4960
6280
7600
Mass(Da)

SS-*p*-SE



SS-*p*-AE



References

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

(2) Ji, Y., Yang, J., Wu, L., Yu, L., and Tang, X. (2016) Photochemical Regulation of Gene Expression Using Caged siRNAs with Single Terminal Vitamin E Modification. *Angew Chem Int Ed Engl 55*, 2152-6.

(3) Pan, J.-f., Yuan, H.-f., Guo, C.-a., Liu, J., Geng, X.-h., Fei, T., Li, S., Fan, W.-s., Mo, X.-m. *et al.* (2015) One-step cross-linked injectable hydrogels with tunable properties for space-filling scaffolds in tissue engineering. *RSC Adv. 5*, 40820-40830.