

## Supporting Information

# NIR Light Controlled Photorelease of siRNA and its Target Intracellular Delivery Based on Upconversion Nanoparticles

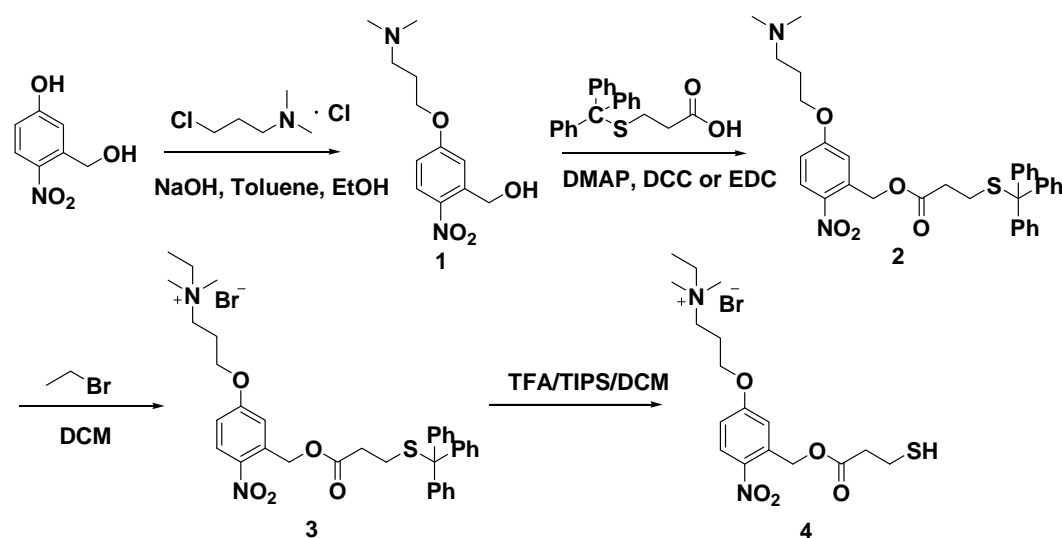
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### Materials and General methods:

**Materials:** Anhydrous solvents for organic synthesis were purchased from Aldrich and stored over activated molecular sieves (4 Å). All reagents were purchased from Aldrich or Fluka. Mal-dPEG<sup>TM</sup><sub>2</sub>-NHS ester linker was purchased from Quanta Biodesign. Unless noted otherwise, commercially available reagents were used without further purification. Thin-layer chromatography (TLC) was performed on glass plates pre-coated with silica gel 60F-254. The PBS buffer for siRNA test was autoclaved at 121 °C for two times. Alexa Fluor@546 labeled siRNA from 1<sup>st</sup> BASE. EGFP siRNA, from Life Technologies

**Instruments:** <sup>1</sup>H NMR spectra were recorded on a JEOL 400 MHz spectrometer. Chemical shifts (δ, ppm) were reported using TMS as an internal reference. EI mass spectra were performed on a Thermo Polaris Q. TEM images were recorded on an FEI EM208S Transmission Electron Microscope (Philips) operated at 100 kV. The size distribution of UCNPs was measured using photon correlation spectroscopy (Brookhaven Instruments Corporation). UV-Vis absorption spectroscopy was performed using a 10-mm path quartz cell on a Beckman coulter DU800 spectrometer. Fluorescence studies were carried out on a Varian Cary Eclipse fluorescence spectrophotometer. Photo-irradiation experiments were carried out using a MDL-H-980 nm laser (5.6 W/cm<sup>2</sup>) and a UV lamp (Blak-Ray, B-100AP/R, 100 w/365 nm, intensity: 8.9 mW/cm<sup>2</sup>).<sup>1, 2, 3</sup>



**Scheme S1** Schematic illustration of the synthesis processes of photocaged linker **4**.

### *Preparation of Compound 1*

5-Hydroxy-2-nitrobenzyl alcohol (340 mg, 2 mmol) and 2-(dimethylamino)propyl chloride hydrochloride (380 mg, 2.4 mmol) were dissolved in DMF (20 mL). Subsequently, sodium carbonate (424 mg, 4 mmol) were added. The reaction mixture was allowed to warm to 80 °C and stirred for 24 hours. After reaction, the DMF solvent was removed under a reduced pressure. The residue was dissolved in ethyl acetate (EA) and washed thoroughly with sodium bicarbonate solution, water and brine. The organic layer was dried and evaporated and purified by column (eluent: DCM/methanol =95:5). Yield 165.1mg (65%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ(ppm): 8.18 (d, J=8.0 Hz, 1H), 7.44 (s, 1H), 7.0 (d, J=7.2Hz, 1H), 4.87 (s, 2H), 4.35 (t, J=7.2Hz, 2H), 3.92 (m, 2H), 2.93 (s, 6H), 2.47 (t, J=8.4 Hz, 2H).

### *Preparation of Compound 2*

3-mercaptopropanoic acid (233.5 mg, 2.2 mmol) was added to the solution of chloro triphenylmethane (556 mg, 2 mmol) in 1.0 ml of anhydrous dichloromethane (DCM). Then trifluoroacetic acid (TFA, 1.0 ml) was added. The solution was stirred for 2 hours under nitrogen condition. The reaction was quenched by 1N NaOH (3 mL). The product was collected and washed by brine (10 mL×3) and dried over anhydrous sodium sulfate. The residue was purified by flash chromatography on silica gel with DCM/MeOH (95 : 5) as eluent to addord 598 mg product (yield: 86%).

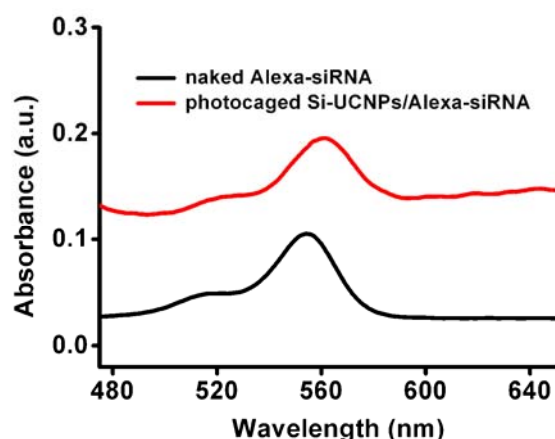
Trityl protected 3-mercaptopropanoic acid (70 mg, 0.2 mmol) was dissolved in dry THF (4 mL) with ice-bath. The dicyclohexylcarbodiimide (DCC, 53 mg, 0.2 mmol) and 4-dimethylaminopyridine (DMAP, 10 mg) were added. Finally, compound **1** was added to the reaction mixture. After the reaction finished indicated by TLC, the formed precipitate was removed by filtration and the filtrate was collect. The organic solvent was removed and the residue was purified by silica gel column (eluent: DCM:MeOH=95:5). Yield 39 mg (36.8 %). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ(ppm): 8.19 (d, J=8.0 Hz, 1H), 7.44~7.21 (m, 15 H), 6.99 (s, 1H), 6.90 (s, 1H), 5.50 (s, 1H), 4.07~4.11 (t, J=8 Hz, 2H), 2.51-2.55 (t, J=8 Hz, 2H), 2.34 (s, 2H), 2.31 (s, 6H), 1.98 (m, 2H), 1.24 (m, 2H), MS (EI): m/z calcd for C<sub>34</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>S 584.73, found 585.04 [M]<sup>+</sup>.

### ***Preparation of Compound 3***

Bromoethane (100 μL, 1.3 mmol) and compound **2** (29 mg, 0.05 mmol) were dissolved in DCM (1 mL). The reaction mixture was stirred under dark condition for 2 day at room temperature. Then the excess bromoethane and solvent were removed under *vacuo*. The residue was washed thoroughly with diethyl ether and dried, the produc (26.7mg, 77.6 %) was obtained. <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ(ppm): 8.16~83.18 (d, J=8.8 Hz, 1H), 7.4~7.14 (m, 15H), 7.09 (s, 1H), 7.07 (s, 1H), 5.44 (s, 2H), 4.2 (t, J=6.0 Hz, 2H), 3.51~3.41 (m, 4H), 3.30 (s, 6H), 2.49 (m, 2H), 2.33 (m, 2H), 2.15 (s, 2H), 1.34 (t, J=7.2 Hz, 3H), MS (EI): m/z calcd for C<sub>36</sub>H<sub>41</sub>N<sub>2</sub>O<sub>5</sub>S<sup>+</sup> 613.79, found 613.22 [M]<sup>+</sup>.

### ***Preparation of Compound 4***

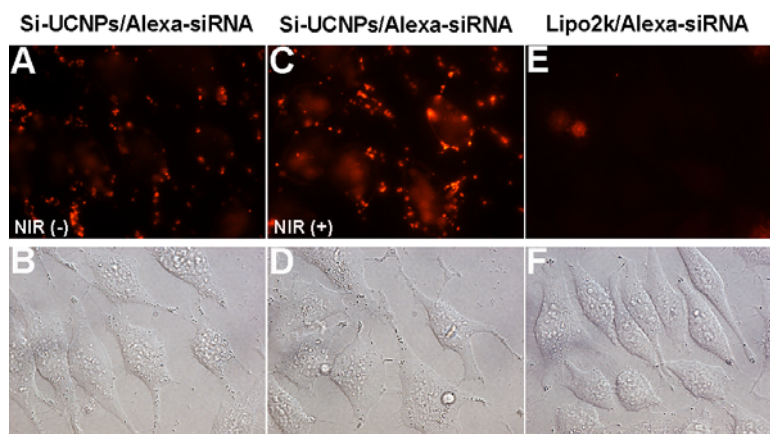
The solution of **3** (15 mg, 0.021 mmol) in DCM (0.5 mL) was cooled with ice bath. To this solution, TFA (1 mL) and triisopropylsilane (50 μL) were added. The mixture was stirred at the same temperature under dark condition for 4 hrs. Then the solvent was removed under reduced pressure. The residue was washed with cold diethyl ether (1 mL × 3). After dried, product **4** was obtained (8 mg, 81%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ (ppm): 8.19 (m, 1H), 7.01~7.02 (m, 2H), 5.51 (s, 2H) 4.2~4.3 (4, 2H), 3.54~3.41 (m, 4H), 3.1 (s, 6H), 2.85 (s, 2H), 2.73 (m, 2H), 2.35 (s, 2H), 1.35 (m, 2H), MS (EI): m/z calcd for C<sub>17</sub>H<sub>27</sub>N<sub>2</sub>O<sub>5</sub>S<sup>+</sup> 371.47, found 371.24 [M]<sup>+</sup>.



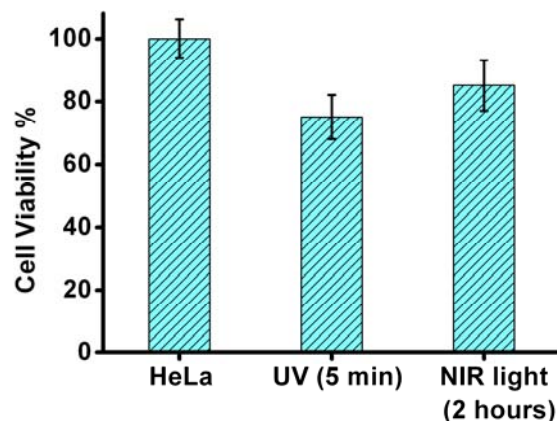
**Fig. S1** UV absorbance spectra of Alexa-siRNA and Si-UCNPs/Alexa-siRNA complex. (1  $\mu$ M).

### *Agarose gel electrophoresis*

The agarose gels were prepared with 2% agarose in Tris-acetate-EDTA buffer (TAE buffer). All of the samples (1  $\mu$ M Alexa-siRNA solution; Si-UCNPs/Alexa-siRNA complex suspended in PBS; supernatant after removal of the complex; washing solution of the complex) were suspended in buffer (5  $\mu$ L sample solution + 5  $\mu$ L Loading Buffer) and added to agarose gel subsequently. The gel electrophoresis was carried out at 100V for 25 minutes and then for gel imaging.



**Fig. S2** (A) and (B), cells incubated with Si-UCNPs/Alexa-siRNA complex before NIR light irradiation. (C) and (D), cells incubated with Si-UCNPs/Alexa-siRNA after NIR light irradiation. (E) and (F), cells treated with standard Lipo2k/Alexa-siRNA. The concentration of Alexa-siRNA was 25 nM.



**Fig. S3** Light cytotoxicity of HeLa with UV light (365 nm) and NIR light (980 nm) irradiation.

#### ***Light cytotoxicity***

The light cytotoxicity was carried out using a standard methyl thiazolyltetrazolium (MTT) assay. HeLa was seeded in a 96-wells plates with a density of  $1 \times 10^4$  per well in culture medium. The medium was changed with fresh DMEM medium after 24 hours incubation. The cells were treated with light irradiation (UV or 980 nm light) for different time. The cytotoxicity activity was evaluated by standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

#### ***Cell viability assay***

The cell viability measurements were also performed on the basis of the standard MTT assay. Similarly, HeLa was seeded in a 96-wells plates with a density of  $1 \times 10^4$  per well in DMEM medium. After 24 hours incubation, the medium was changed with fresh culture and the cells were treated with different substrates: Alexa-siRNA only, Lipo2k/Alexa-siRNA, Si-UCNPs/Alexa-siRNA without NIR light irradiation and Si-UCNPs/Alexa-siRNA with NIR light irradiation (2 hours), respectively. All the incubation time is 1 hour and the concentration of Alexa-siRNA and UCNPs was 25 nM and 0.03 mg/mL. The cells without any treatment were served as control.

For gene silencing experiment, the cell viability measurements were evaluated by using the same protocol. All of the HeLa cells were transfected with EGFP plasmid except the control cells. Then the cells were incubated with different siRNA substrates: Lipo2k/siRNA (5 hours incubation), Si-UCNPs/EGFP siRNA without light irradiation (5 hours incubation) and Si-UCNPs/EGFP siRNA with 3 hours incubation time followed by light irradiation (2 hours). The medium was

replaced with fresh culture medium and conducted the MTT assay.

1. C. J. Carling, F. Nourmohammadian, J. C. Boyer and N. R. Branda, *Angew. Chem., Int. Ed.*, 2010, **49**, 3782-3785.
2. L. Poon, W. Zandberg, D. Hsiao, Z. Erno, D. Sen, B. D. Gates and N. R. Branda, *ACS Nano*, 2010, **4**, 6395-6403.
3. K. Yehl, J. P. Joshi, B. L. Greene, R. B. Dyer, R. Nahta and K. Salaita, *ACS Nano*, 2012. DOI:10.1021/nn303426.