Electronic Supplementary Information for

Shell-detachable nanoparticles based on a light-responsive amphiphile for enhanced siRNA delivery

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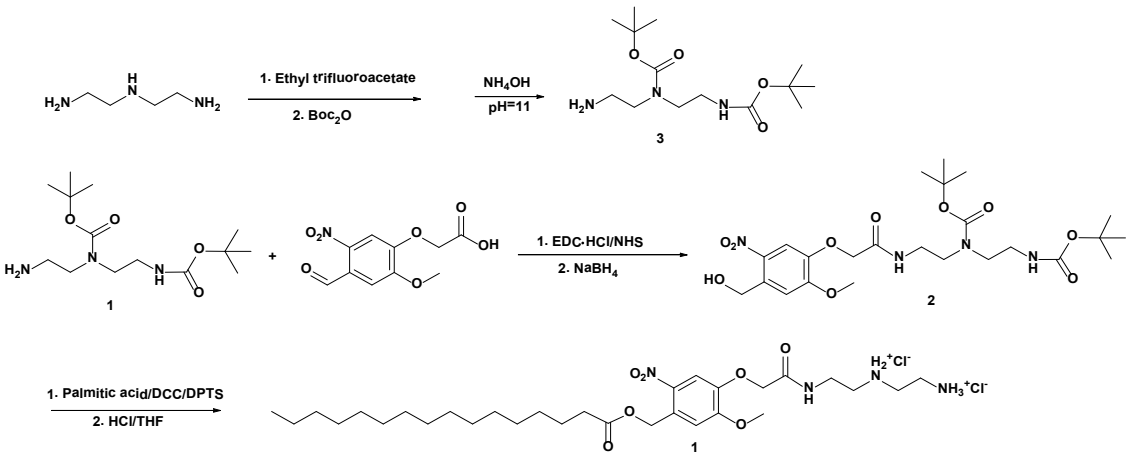
Materials. Diethylenetriamine (DETA, Sinopharm Chemical Reagent Co., Ltd., China) was dried over CaH_2 overnight and distilled before use. Palmitic acid (Sinopharm Chemical Reagent Co., Ltd., China) was purified by recrystallization from hot ethanol. Ethyl trifluoroacetate, (3-dimethylaminopropyl) ethyl-carbodiimidmonohydrochloride (EDC·HCl), di-tert-butyldicarbonate (Boc_2O) and sodium borohydride were purchased from Aladdin Industrial Inc. (Shanghai, China) and used without purification. *N*-Hydroxysuccinimide (NHS) and *N, N′*-Dicyclohexylcarbodiimide (DCC) were purchased from Shanghai Medpep Co., Ltd. (Shanghai, China) and used without purification. *N′,N*-Dimethylformamide (DMF), dichloromethane (DCM) and methanol were dried over CaH_2 overnight and distilled before use. Tertrahydrofuran (THF) was dried over a Na-K alloy and used just before use. 2-(4-formyl-2-methoxy-5-nitrophenoxy) acetic acid was synthesized as reported.[1] 4-(dimethylamino)-pyridinium *p*-toluenesulfonate (DPTS) was prepared as previously reported.[2] FAM-labeled siRNA (FAM-siRNA), siRNA with a scrambled sequence (si*N.C.*) and siRNA targeting luciferase mRNA (si*Luc*) were obtained from Suzhou Ribo Life Science Co. (Jiangsu, China). All other chemicals were of reagent grade and used without further purification.

Characterization. ^1H NMR spectra were recorded on a Bruker AV400 NMR spectrometer using $CDCl_3$ or DMSO-d_6 as the solvents. The UV-Vis absorbance

change in the solution after UV irradiation (λ_{max} = 365 nm, EXFO Omnicure™1000, Canada) was measured on a UV-2802 PC (UNICO, China) spectrometer. Dynamic light scattering (DLS) measurements were performed in aqueous solution using a Malvern Zetasizer Nano ZS90 with a He-Ne laser (633 nm) and 90° collecting optics. The data were analyzed by Malvern Dispersion Technology Software 4.20. Electrospray ionization mass spectrometry (ESI-MS) was performed on a Proteome X-LT Q (ThermoFisher Scientific, USA). Transmission electron microscopy (TEM) observation was performed on a JEOL JEM-2100F transmission electron microscope (JEOL Co., Ltd., Tokyo, Japan) with an accelerating voltage of 200 kV.

Synthetic procedures



Scheme S1. Synthetic route to the UV-cleavable amphiphile **1**.

Synthesis of compound 3. Selective protection of one amino group of DETA was processed by reaction with ethyltrifluoroacetate.[3] DETA (3.09 g, 1.0 eqv) was dissolved in 140 mL of methanol, and the solution was cooled to -78°C, then ethyl trifluoroacetate (4.23 g, 1.0 eqv) was added dropwise over a period of 30 min at -78°C. Stirring continued for 1 h at 0°C to complete the reaction. The remaining two amino groups were protected with Boc_2O without further purification. Boc_2O (16.34 g, 2.5 eqv) was dissolved in 30 mL of methanol, the solution was added dropwise over a period of 30 min, and the mixture was stirred at 0°C for 1 h and then overnight at room temperature. After solvent removal by a rotary evaporator, the residue was

redissolved in DCM and purified by washing with saturated sodium bicarbonate and sodium chloride solution in sequence, and the organic phase was collected and dried with magnesium sulfate. After filtration, the filtrates were concentrated into 10 mL and then added into a 15 times greater volume of n-hexane, then kept at -20°C overnight. The intermediate was obtained by filtration as white powder. The trifluoroacetamide protecting group was then removed by adding 30 mL of 25% ammonia solution (pH of the mixture was 11), then the solution was stirred for 16 h at room temperature. After removing the solvent under reduced pressure, the residue was further purified by chromatography over silica gel (DCM/MeOH/NH_4OH = 100/10/1, v/v/v). The final product was obtained as yellow oil (yield: 40%). ^1H NMR (400 M Hz, $CDCl_3$, ppm): δ 5.33 (s, 1H), 3.49 (t, 2H), 3.28 (t, 4H), 2.85 (t, 2H), 1.46 (s, 18H). MS (ESI): m/z (relative intensity, %) = 304.10 (100) $[M+H]^+$, 606.82 (38) $[2M+H]^+$.

Synthesis of compound 2. Compound **3** (0.50 g, 1.0 eqv), 2-(4-formyl-2-methoxy-5-nitrophenoxy) acetic acid (0.51 g, 1.2 eqv) and NHS (0.37 g, 2.0 eqv) were dissolved in 10 mL of DMF/DCM = 1/1 (v/v), then EDC·HCl (0.61g, 2.0 eqv) in 10 mL of DCM was added dropwise over a period of 30 min at 0°C, and further stirred at room temperature for 24 h to complete the reaction. The solvents were removed under reduced pressure followed by redissolution in DCM. Then, the organic phase was washed with saturated sodium bicarbonate and sodium chloride solution in sequence, and finally dried over magnesium sulfate. After filtration, the filtrate was concentrated under reduced pressure and the product was obtained as a waxy solid (0.87 g, yield: 98%).

The waxy solid was dissolved in dried THF (5 mL) and methanol (20 mL) and chilled to 0°C. Then, $NaBH_4$ was added to the solution in several portions. After stirring at 0°C for 10 min, the mixture was further stirred at room temperature for 6 h. 10 mL of 0.1 mol/L NaOH was added to adjust the pH to 11, and the solution was further stirred overnight. After removal of the solvent by a rotation evaporator, the

product was dissolved in DCM and then washed with distilled water, saturated sodium bicarbonate and sodium chloride solution in sequence, and finally dried over magnesium sulfate. After filtration, the filtrate was concentrated under reduced pressure and the product was purified by chromatography over silica gel (DCM/MeOH = 98/2, v/v). The final product was obtained as a light yellow foamy solid (0.38 g, yield: 40%). ^{1}H NMR (400 M Hz, DMSO-d_6, ppm): δ 7.75 (s, 1H), 7.27 (s, 1H), 5.00 (d, 2H), 4.56 (s, 2H), 4.01 (s, 3H), 3.20-3.54 (m, 8H), 1.44 (s, 18H). MS (ESI): m/z (relative intensity, %) = 542.80 (100) $[M+H]^+$, 1084.62 (17) $[2M+H]^+$, 1101.74 (35) $[2M+NH_4]^+$.

Synthesis of amphiphile 1. Palmitic acid (0.163 g, 1.0 eqv), compound **2** (0.380 g, 1.1 eqv) and DPTS (0.094 g, 0.5 eqv) were dissolved in 5 mL of DCM, then DCC (0.263 g, 2.0 eqv) dissolved in 5 mL of DCM was added dropwise into the mixture and stirred at room temperature for 24 h. After filtration to remove the precipitate, the filtrate was diluted with more DCM and washed with a saturated sodium chloride solution. The organic layer was dried over magnesium sulfate. After filtration, the filtrate was concentrated under reduced pressure and the product was purified by chromatography over silica gel (DCM/MeOH = 98/2, v/v). The product was obtained as a light yellow solid (0.40 g, yield: 80%). The product (0.135 g) was dissolved in 5 mL of anhydrous THF followed by the addition of 5 mL of a HCl/THF solution (11 M) at 0°C and then stirred for 4 h. After removing the solvent under reduced pressure, the residue was washed by ether three times. The final product was obtained as a white powder (84 mg, yield: 75%). ^{1}H NMR (400 M Hz, DMSO-d_6, ppm): δ 7.73 (s, 1H), 7.23 (s, 1H), 5.38 (s, 2H), 4.70 (s, 2H), 3.94 (s, 3H), 3.45 (m, 2H), 3.05-3.26 (m, 6H), 2.39 (t, 2H), 1.55 (m, 2H), 1.23 (m, 24H), 0.85 (t, 3H). MS (ESI): m/z (relative intensity, %) = 581.38 (100) $[M+H]^+$.

Preparation of UV-sensitive nanoparticles (USNPs) and USNP/siRNA complexes. USNPs were prepared by the dialysis method. In brief, 10 mg of amphiphile **1** was dissolved in 1 mL of DMSO and then added slowly into 8 mL of ultra-purified water

(Millipore, 18.2 MΩ, Bedford, MA) with moderate stirring at 0°C. After stirring at this temperature for 0.5 h, the solution was transferred onto a dialysis membrane (Spectra/Por®, Float-A-Lyzer, with molecular weight cut-off of 3.5 kDa) and dialyzed against ultra-purified water at room temperature to completely remove the organic solvent. The final concentration of USNPs was 0.68 mg/mL.

For siRNA loading, the USNP aqueous suspension was diluted to different concentrations and mixed with siRNA solution at a concentration of 1 μM. The mixture was mixed gently by pipetting and allowed to stand at room temperature for 20 min before subsequent experiments.

Gel retardation assay. USNP/siRNA complexes were prepared at different N/P ratios as described above. Then, the complexes were electrophoresed on a 1% agarose gel in Tris-borate-EDTA buffer (TBE buffer, 89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3) at 110 V for 10 min, and visualized under a UV illuminator (Tanon GIS System, Shanghai, China) with ethidium bromide staining.

Photolysis experiments of USNPs and USNP/siRNA complexes.

a. The USNP solution was diluted to 0.2 mg/mL. The solution was then subjected to UV irradiation (λ_{max} = 365 nm, 10 mW/cm^2). At predetermined time intervals, the solution was withdrawn and its UV-vis absorption spectroscopy was measured.

b. The USNP/siRNA complexes at an N/P ratio of 25 were then subjected to UV irradiation (λ_{max}= 365 nm, 10 mW/cm^2). At predetermined time intervals, the complexes were withdrawn and analyzed by the gel retardation assay as described above to determine the release of siRNA.

In vitro cell-based assays.

a. *Cellular uptake.* MDA-MB-231 cells were plated in 24-well plates at a density of 5×10^4 cells/well and incubated in complete DMEM medium for 24 h. The cells were washed and treated with USNP/FAM-siRNA at a FAM-siRNA dose of 200 nM. After incubation at 37°C for 3 h or 5 h in serum-free DMEM medium, the cells were trypsinized, washed with PBS twice, resuspended in 200 μL of PBS and subjected to

flow cytometric analyses on a BD FACSCalibur flow cytometer (BD Bioscience, Bedford, MA).

For microscopic observation, MDA-MB-231 cells, seeded onto coverslips, were incubated with USNP/FAM-siRNA at a FAM-siRNA dose of 200 nM for 3 h in serum-free DMEM medium. After receiving 0 or 300 s of UV irradiation, the cells were further incubated for 1 h and were then washed twice with PBS and fixed with 4% paraformaldehyde (pH 7.4 in PBS) at room temperature for 15 min. Washed twice with PBS and then incubated in 0.1% Triton X-100 in PBS for 3 min. Staining with Alexa Fluor®568 phalloidin with PBS containing 1% BSA for 20 min. Washed twice with PBS again and added DAPI staining solution, incubate for 5 min, rinsed two times in PBS. The slides were mounted using one drop of Fluoromount™ Aqueous Mounting Medium and observed with a Zeiss LSM 710 laser confocal scanning microscope imaging system (Zeiss, Heidenheim, Germany) with a 63× objective.

b. *siRNA transfection.* For luciferase gene silencing, stable luciferase-expressing MDA-MB-231 (MDA-MB-231-luciferase) cells were plated at a density of 5×10^4 cells/well in a 24-well plate and incubated for 24 h in complete DMEM medium. Cells were transfected with siRNA using USNP/si*Luc*, free si*Luc* and USNP/si*N.C.* at an siRNA dose of 200 nM. MDA-MB-231-luciferase cells were transfected for 3h. After replacing the media with fresh culture media, some wells were treated with UV light irradiation (λ_{max} = 365 nm, 10 mW/cm^2) for 300 s. The cells were harvested at 48 h after transfection. For luciferase silencing efficiency analyses, cells were washed and lysed with a reporter lysis buffer (Promega, San Luis Obispo, USA) and luciferase activity was measured for 10 s on a Veritas Microplate Luminometer (Model 9100-102, Turner BioSystems, USA) using a Promega luciferase assay kit according to the standard protocol provided by the manufacturer. Light units (RLU) were normalized to protein concentrations in the cell extracts, which were measured using a bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, USA).

c. *MTT cytotoxicity assay.* Cells were seeded in 96-well plates at 1×10^4 cells/well and incubated at 37°C in 5% CO_2 atmosphere in complete DMEM medium for 24 h. Cells were then treated with USNPs at various concentrations in complete DMEM medium for 48 h. Following that, 25 μL of MTT stock solution (5 mg/mL in PBS) was added to each well to achieve a final concentration of 1 mg/mL, with the exception of the blank wells, to which 25 μL of PBS was added. After incubation for another 2 h, 100 μL of extraction buffer (20% sodium dodecyl sulfate in 50% DMF, pH 4.7) was added to the wells and incubated overnight at 37°C. The absorbance was measured at 570 nm using a Bio-Rad 680 microplate reader (Hercules, CA). The cell viabilities were normalized to those of cells cultured in complete culture medium.

Results

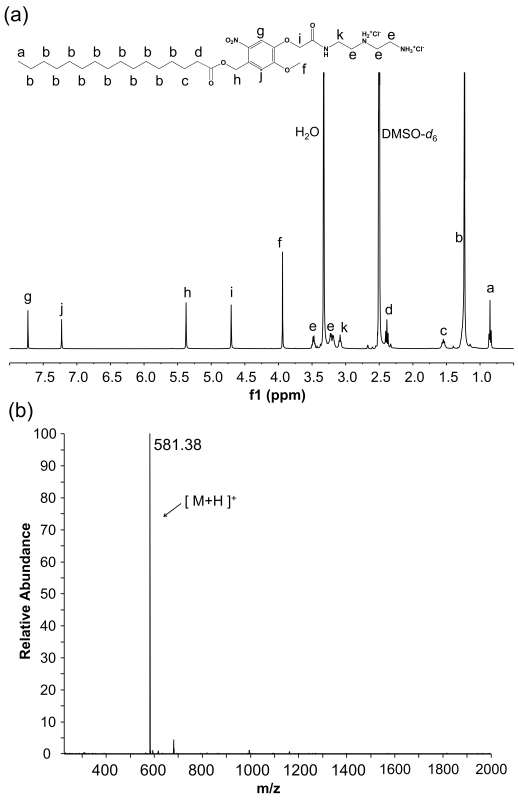


Fig. S1. (a) ^{1}H NMR (DMSO-d_6) and (b) ESI-MS data of amphiphile **1**.

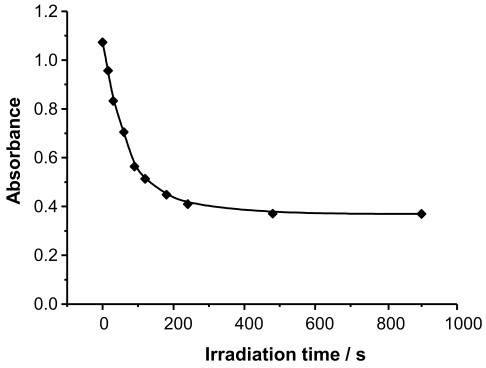


Fig. S2. Time-dependent change in UV-vis absorbance at 345 nm of USNPs after UV irradiation.

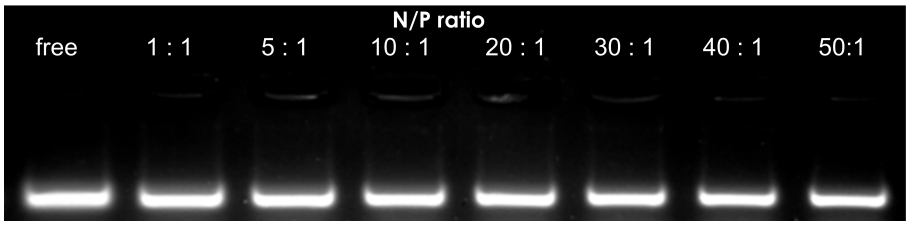


Fig. S3. Agarose gel analysis of the siRNA migration of the DETA·HCl/siRNA complexes at N/P ratios varying from 1/1 to 50/1 in TAE buffer.

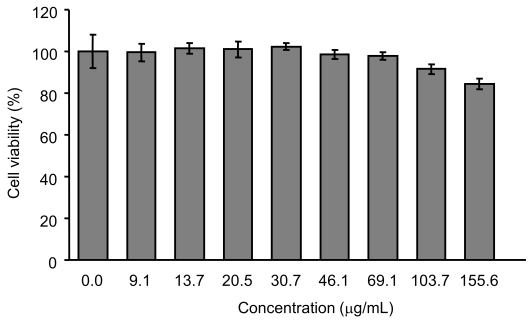


Fig. S4. MTT cytotoxicity assay of USNPs at different concentrations against MDA-MB-231 cells after 48 h incubation.

References

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3. S. Srinivasachari, K. M. Fichter and T. M. Reineke, *J. Am. Chem. Soc.*, 2008, **130**, 4618-4627.