

Supporting Information

Highly Efficient Photocontrolled Targeted Delivery of siRNA by a Cyclodextrin-Based Supramolecular Nanoassembly

*Feng-Qing Li, Qi-Lin Yu, Yao-Hua Liu, Hua-Jiang Yu, Yong Chen, Yu Liu**

Contents

1 Materials and methods

1.1 Synthesis of *trans*-G

1.2 Synthesis of HA- α -CD

1.3 Synthesis and characterization of the assemblies.

2 Confocal microscopy

3 Gene silencing and cell viability assays

1 Materials and methods

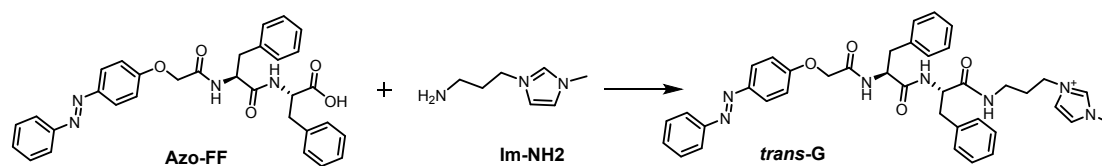
All chemical reagents were commercially available unless noted otherwise. AZO-FF and Im-NH₂ were synthesized according to the reported procedures^[1]. Free **trans-G** was synthesized by amide condensation reaction of AZO-FF and Im-NH₂ (see 1.1).

NMR spectra were recorded on a Bruker 400 MHz instrument at 20 °C. The UV light irradiation experiment was carried out using a ZF-7A lamp (365 nm, 8 W), and the visible light irradiation experiment ($\lambda > 420$ nm) was carried out using a CEL HXF300 xenon lamp with cutoff filter. UV/Vis spectra were recorded on a Shimadzu UV-3600 spectrophotometer in a quartz cell (light path 10 mm) equipped with a PTC-348WI temperature controller.

TEM images were acquired by a high-resolution transmission electron microscope (Philips Tecnai G2 20 S-TWIN microscope) operating at an accelerating voltage of 200 keV. The samples were prepared by placing a drop of solution onto a carbon-coated copper grid and air-dried. SEM images were acquired by a JEOL JSM-7500F scanning electron microscope (operating voltage, 5 kV). The samples were prepared by placing a drop of solution onto a silicon substrate and air-dried. All the samples were sputtered with platinum for 120 s under vacuum. Typically, the concentration of the free **trans-G**, **trans-G/α-CD** samples was diluted into 3×10^{-5} mol L⁻¹ in TEM and SEM. The concentration of **trans-G/HA-α-CD** and the ternary supramolecular assembly **trans-G/HA-α-CD/siRNA** in TEM and SEM was ca. 8 μM.

The **siRNA** (GAPDH-homo, purchased from Shanghai GenePharma Co., Ltd.) was stored at 4 °C. The sequences of siRNA are shown as follow: sense UGA CCU CAA CUA CAU GGU UTT and anti-sense AAC CAU GUA GUU GAG GUC ATTT. The initial concentration of siRNA was 100 μM (dissolved in dH₂O). After diluted into the experiment concentration (1 μM), the vial containing siRNA solution was put under 37 °C for 15 minutes.

1.1 Synthesis of *trans*-G



Synthesis of *trans*-G

To a dry DMF solution (20 mL) of AZO-FF (100 mg, 0.18 mmol, 1.0 equiv), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC, 70 mg, 0.36 mmol, 2.0 equiv) and 1-hydroxybenzotriazole (HOBT, 49 mg, 0.36 mmol, 2.0 equiv) was added at 0-5 °C under inert atmosphere. After 10 min, Im-NH₂ (100 mg, mmol, 1.0 equiv) was added, and the resulting solution was stirred at room temperature under inert atmosphere for 12 h. Then the solution was filtrated and purified by prep-HPLC (Waters Xbridge BEH C18 reversed phase chromatography column, 100 * 25 mm * 5 um) with the mobile phase of water (0.2 % trifluoroaceticacid)-acetonitrile to give *trans*-G (80 mg) as a yellow solid (yield 65%). ¹H NMR spectrum (400 MHz, CDCl₃, 298 K), δ 8.52(s, NH, 2H), 7.60 - 7.58 (d, 2H), 7.35-7.20 (m, 14H), 6.98 - 6.94 (m, 3H), 6.66 – 6.64 (m, 3H), 4.13 (s, 3H), 3.96-3.92 (m, 4H), 3.65 (s, 4H), 3.25-3.16 (m, 4H), 2.61 (s, 2H). ¹³C NMR (400 MHz, CDCl₃, 298 K), δ 172.88, 172.56, 172.04, 171.62, 169.45, 168.18, 161.81, 161.44, 159.58, 159.41, 152.64, 147.61, 138.88, 138.50, 137.39, 136.24, 130.70, 129.26, 129.10, 128.70, 127.02, 126.71, 124.76, 123.15, 122.66, 121.68, 114.85, 77.34, 77.23, 77.03, 76.71, 67.06, 56.32, 55.66, 46.73, 37.43, 36.88, 36.31, 34.60, 28.92. MALDI-TOF: *m/z* 672.3296 [M]⁺ (calcd. 672.33).

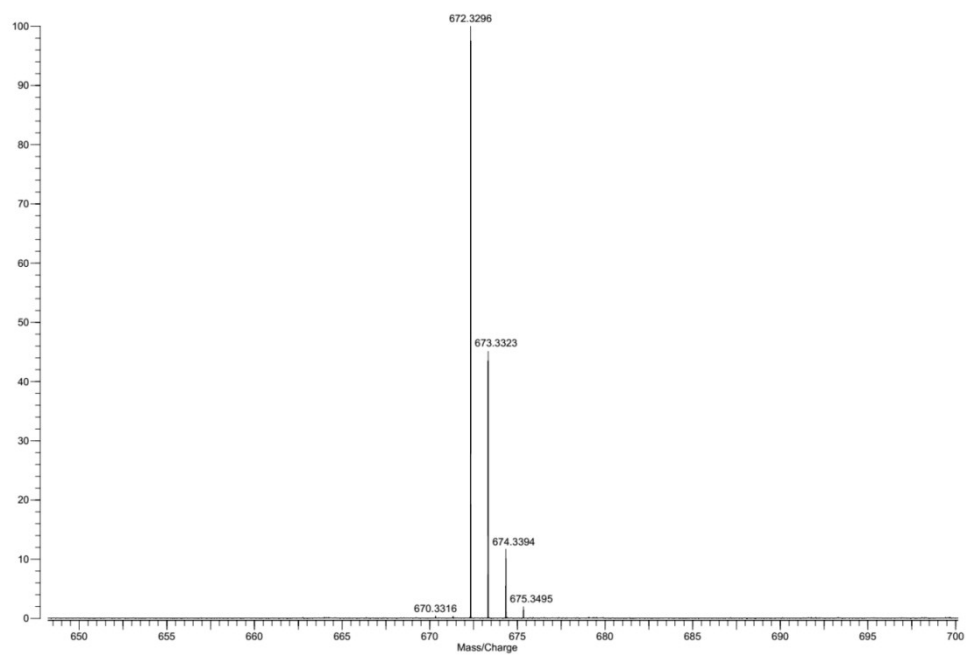


Fig. S1. MALDI-TOF Mass of *trans*-G.

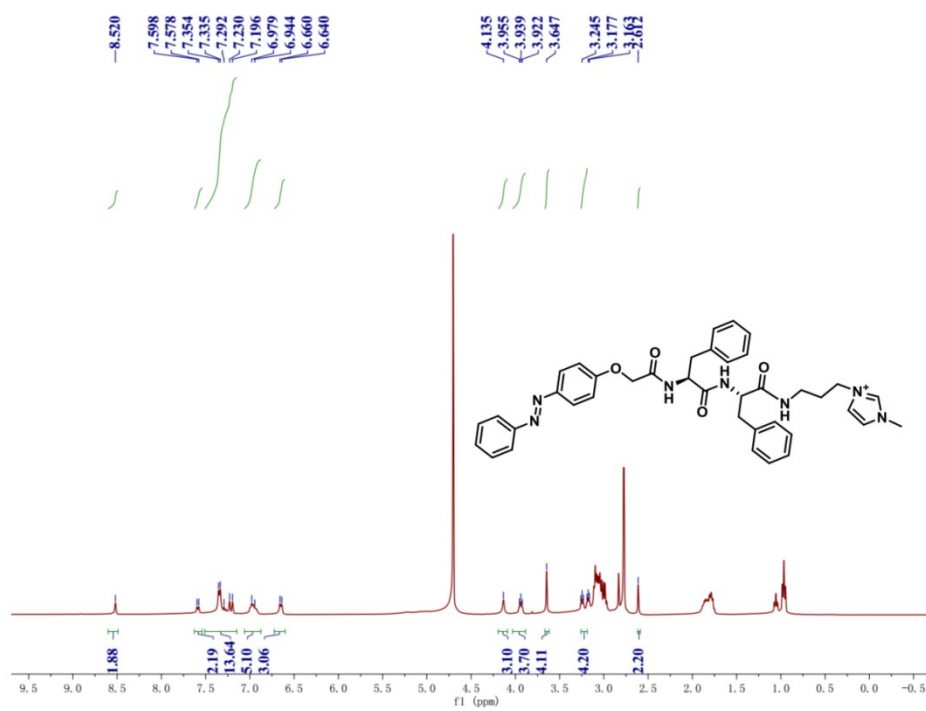


Fig. S2. ¹H NMR spectrum (400 MHz, CDCl₃, 298 K) of *trans*-G.

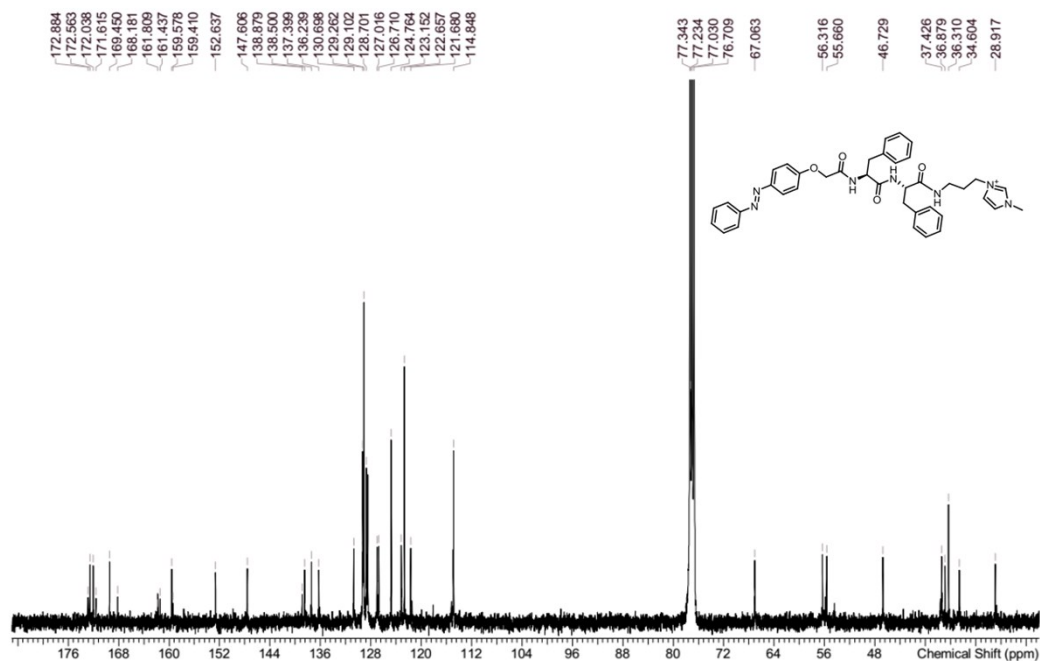


Fig. S3. ^{13}C NMR spectrum (400 MHz, CDCl_3 , 298 K) of *trans*-G.

1.2 Synthesis of HA- α -CD

The sodium hyaluronate (500 mg, Mw = 40000) was dissolved into the PBS solution (150 mL, pH = 7.2). EDC·HCl (835.8 mg, 4.36 mmol) and NHSS (500 mg, 2.3 mmol) was added into the solution and stirred at room temperature for 30 min. Then NH_2 - α -CD (1000 mg, 0.98 mmol) was added and the resulting mixture was stirred at room temperature for 24 h. Subsequently, the solution was dialyzed against pure water for 5 days. After being freeze-dried and dried under vacuum, HA- α -CD (450 mg, 70 % yield,) was obtained as white solid. ^1H NMR (400 MHz, D_2O , 298 K) δ (ppm): 4.97-4.90 (m, 6H), 4.39-4.31 (m, 6H), 3.83-3.34 (m, 69H), 1.86 (s, 9H). By comparing the integration area of the proton signals at 4.35 ppm and 1.86 ppm (assigned to HA protons) with that of the proton signals at 4.9 ppm (assigned to C-1 protons of α -CD), the degree of substitution of α -CD units grafted on HA- α -CD was calculated as 28.7%.

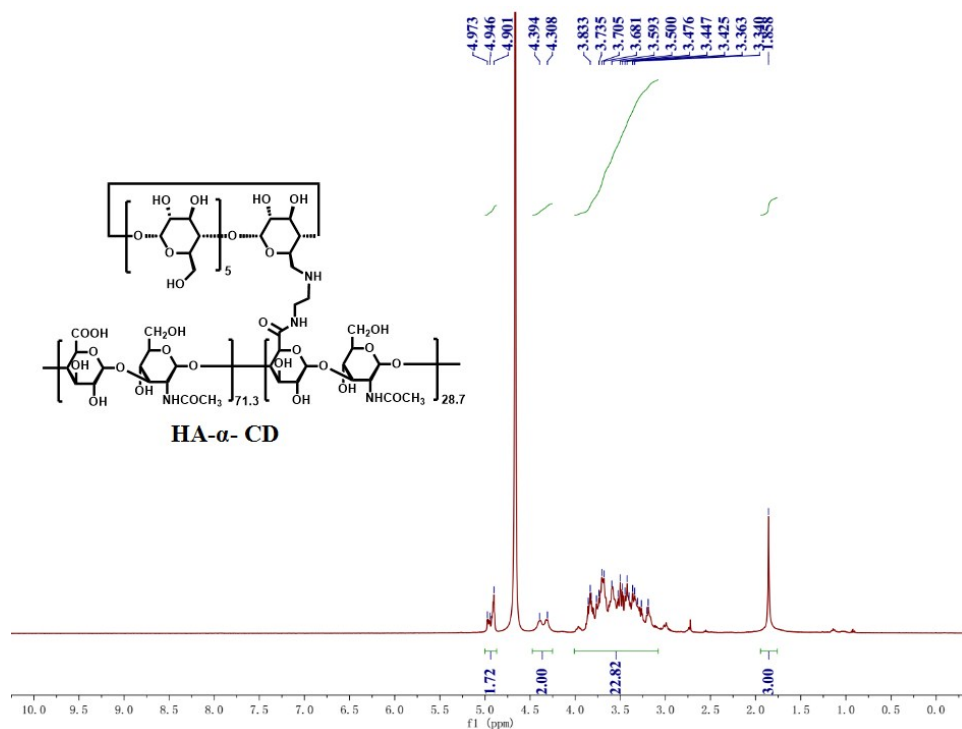


Fig. S4. ^1H NMR spectrum (400 MHz, 298 K) of HA- α -CD in D_2O .

1.3 Synthesis and characterization of the assemblies.

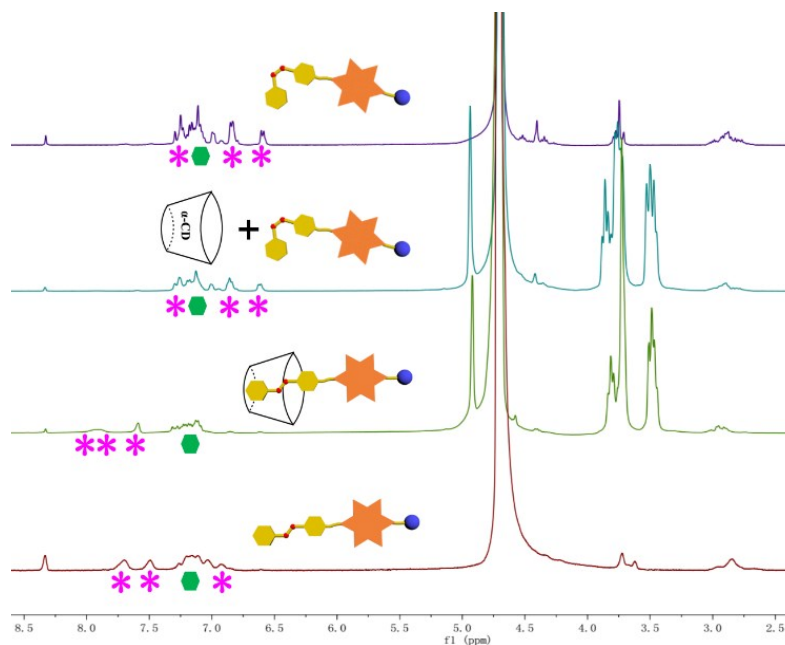


Fig. S5 Partial ^1H NMR spectra of (a) free *trans*-G (2.5×10^{-3} mol L^{-1}), (b) *trans*-G/ α -CD, (c) *cis*-G + α -CD, and (d) free *cis*-G (2.5×10^{-3} mol L^{-1}). The purple asterisks indicate the aromatic protons of the azobenzene moiety, and the green hexagons indicate the aromatic protons of the FF and imidazole moieties.

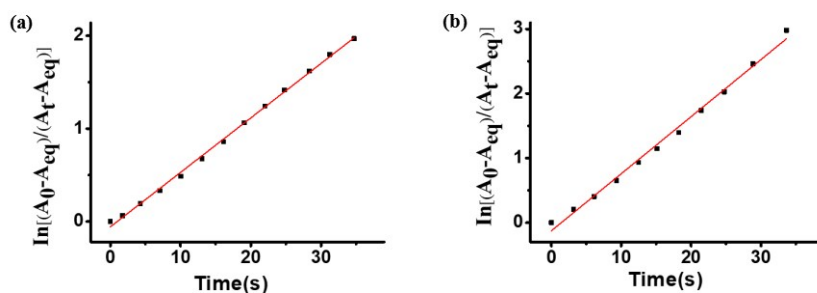


Fig. S6. Determination of the rate constants (k_t) for photoisomerization of (a) *trans*-G and (b) *trans*-G/ α -CD

upon irradiation with UV light at 365 nm.

The ***trans*-G/ α -CD** solution was prepared by the dilution of *trans*-G stock solution in aqueous with α -CD aqueous solution at equal molar ratio, and then the mixture was ultrasonicated for 10 min.

The ***trans*-G/HA- α -CD** solution (160 μ M) solution was obtained by the dilution of *trans*-G stock solution in aqueous with HA- α -CD aqueous solution which possesses equal molar of α -CD units according to the degree of substitution of HA- α -CD, and then the mixture was ultrasonicated for 10 min.

The ***trans*-G/HA- α -CD/siRNA** solution was prepared by dropping siRNA into the freshly prepared *trans*-G+HA- α -CD solution. The resulting *trans*-G/HA- α -CD/siRNA solution was stored at 4 °C.

Free *cis*-G, *cis*-G/ α -CD, *cis*-G/HA- α -CD, and *cis*-G/HA- α -CD/siRNA solution was obtained by ***trans*-G**, ***trans*-G/ α -CD**, ***trans*-G/HA- α -CD**, and ***trans*-G/HA- α -CD** solution irradiated at 365 nm for 3 min, respectively.

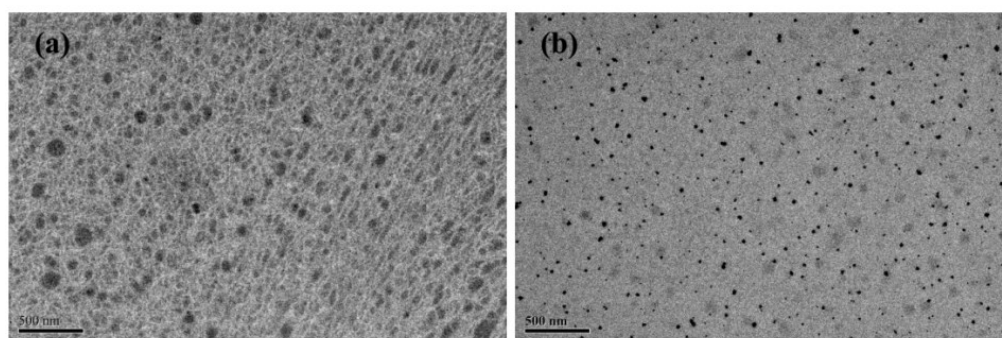


Fig. S7. TEM images of (a) *cis*-G/ α -CD, (b) *cis*-G/HA- α -CD.

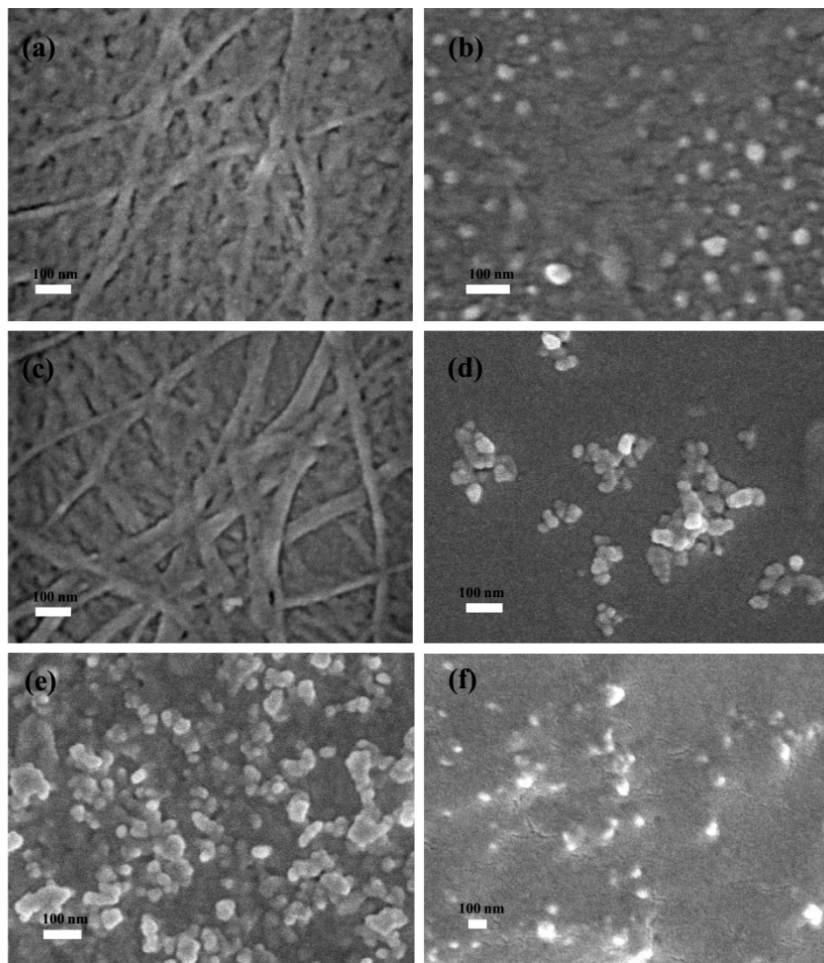


Fig. S8. SEM images of (a) free *trans*-G, (b) free *cis*-G, (c) *trans*-G/ α -CD, (d) *trans*-G/HA- α -CD, (e) *trans*-G/HA- α -CD/siRNA, and (f) *cis*-G/HA- α -CD/siRNA.

2 Confocal microscopy

Human lung adenocarcinoma cells (A549, Cell Resource Center, China Academy of Medical Science, Beijing, China) were seeded in 96-well plates (5×10^4 cells mL⁻¹, 100 μ L per well) for 24 h at 37 °C in 5% CO₂. Then, the cells were incubated with free siRNA (1 μ M), HA- α -CD (160 μ M) + siRNA (1 μ M), *trans*-G (160 μ M) + siRNA (1 μ M), and *trans*-G/HA- α -CD/siRNA (*trans*-G 160 μ M, HA- α -CD 160 μ M, siRNA 1 μ M) for 24 h, respectively. Subsequently, the cells were washed with PBS, fixed and stained with DAPI. The cells were then observed by confocal microscopy (FV1000, Olympus, Japan).

3 Gene silencing and cell viability assays

The A549 tumor cells or NIH3T3 non-cancerous cells were incubated with free *trans*-G, HA- α -CD, *trans*-G/HA- α -CD assembly, *trans*-G + siRNA, HA- α -CD + siRNA and *trans*-G/HA- α -CD/siRNA ternary assembly at the concentrations of host or guest with 20 μ M, 40 μ M, 80 μ M, 160 μ M, and 320 μ M, and with the concentration of siRNA with 1 μ M for 24 h, respectively. The viability of the treated cells was then detected by the CCK-8 Assay Kit (DOJINDO, Japan). Statistically significant difference is indicated with asterisk (* $p < 0.05$).

To evaluate gene silencing efficiency of the assemblies and the control agents, the cells were treated by 80 μ M of the assemblies (containing 1 μ M siRNA) or the control agents. After 24 h of incubation, total mRNAs were isolated from the cells, and expression levels of GAPDH were measured by real-time (RT) PCR using a RT-PCR assay kit (Promega, USA).

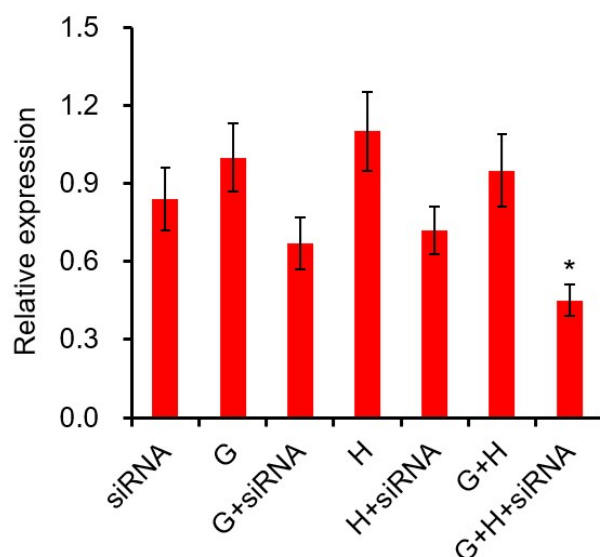


Figure S9. Relative expression of GAPDH in the A549 cells treated by the GAPDH siRNA, G, H, or the assemblies containing the GAPDH siRNA or not. The expression levels of GAPDH were evaluated by real-time PCR method. * indicates significant difference between the G+H+siRNA group and other groups ($P < 0.05$). Noting that gene silencing efficiency of G+H+siRNA reaches to 55%.

Reference

- [1] (a) P. Bhunia, E. Hwang, M. Min, J. Lee, S. Seo, S. Some and H. Lee, *Chem. Commun.* 2012, **48**, 913-915; (b) W. Zhang, Y. Chen, J. Yu, X.-J. Zhang and Y. Liu, *Chem. Commun.* 2016, **52**, 14274-14277.