Supporting Information

G-quadruplex/protoporphyrinIX-Functionalized Silver Nanoconjugates for Targeted Cancer Cell Photodynamic therapy

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Materials and methods

1. Chemicals and materials

Tris(β-chloroethyl) phosphate (TCEP) was purchased from Fluka (Buchs, Switzerland). Glutathione and all chemicals of analytical grade were used as received without further purification.The oligodeoxynucleotides used in the present study were SH-AS1411, an antiproliferative G-rich oligodeoxynucleotides, whose sequence is 5'-d(GGTGGTGGTGGTTGTGGTGGTGGTGGTTTSH)-3', and were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). 3-[4, 5-dimethylthiazolyl-2]-2, 5-diphenyltetrazolium bromide were obtained from Sigma–Aldrich (USA). Phosphate buffered solution (PBS) were prepared by 10mM phosphate (NaH_2PO_4 and Na_2HPO_4), pH=7.4. The PBS buffer was used to rinsing suspension of HeLa cells. All the solutions were prepared by using distilled water and stored at 4 °C before use. HeLa cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in DMEM supplemented with 10% standard fetal bovine serum (Defined FBS) (HyClone Laboratories, UT) at 37 °C and in 5% CO_2. Glass chamber slides (14 mm bottom well) were purchased from Hangzhou Sanyou Biotechnology Co. Ltd. (Hangzhou, China).

2. Apparatus

Fluorescence measurements were recorded at room temperature using a LS 55 luminescence spectrometer (Perkin-Elmer). CD signal was performed by JASCO-820 Circular Dichroism spectrometer. The sample for cell imaging was obtained by fixing the bound cells using PAAs on a 35-mm tissue culture dish (World Precision Instruments) and the result of fluorescence images were acquired using LEICA TCS SP2 laser scanning confocal microscope (LSCM) (Germany) with a 100 x oil immersion objective. TEM images were obtained with a FEI TECNAI G^2 transmission electron microscope (Netherlands) operating voltage of 120 kV. The samples for TEM characterization were prepared by placing a drop of colloidal solution on carbon-coated copper grid and dried at room temperature. AFM image was collected by using a SPI3800N microscope (Seiko Instruments Industry Co., Tokyo, Japan) (Seiko Instru-ments, Inc.) operating in the tapping mode with standard silicon nitride tips. Typically, the surface was scanned at 1 Hz with the resolution of 256 lines/image. The result of MTT was got from EL808 ultramicroplate reader (Bio-TEK Instrument, Inc., Winooski, VT, USA).

3. Synthesis and mofication of AgNPs

AgNPs of 5 nm diameter were synthesized by the glutathione reduction method [1]. SH-AS1411 was activated with two equivalents of tris(2-carboxyethyl) phosphine hydrochloride (TCEP). TCEP-activated SH-AS411 and AgNPs were mixed and stirred at room temperature for 24 h or longer, and Tris-HCI buffer was added to the solution to give a pH value of 7.4. The solution was left at room temperature for another 24 hours at 4°C. The DNA-functionalized AgNPs were purified by centrifugation to remove the supernatant before use [2]. The purified DNA-functionalized AgNPs were dissolved in a buffer containing 100 mM KCl, 20 mM Tris-HCI buffer (pH = 7.4) at a concentration of 10 μM and allowed to dissolve over several hours to form the G-quadruplex DNA structure. Then, the PPIX was loaded on the G-quadruplex DNA structure (refered as PAAs) as the targeted PDT.

4. Bioimaging

HeLa cells were incubated with PAAs. HeLa cells were plated onto 35 mm glass chamber slides. Stock solutions of PAAs in PBS buffer were prepared at concentrations of 10 μM. Diluted solutions in complete growth medium were then freshly prepared and placed over the cells for 2-3 h. All cells were washed with PBS buffer (3×) at room temperature. After that, cells were scanned by LEICA TCSSP2 laser scanning confocal microscope (LSCM). HeLa cells (10^6 cells per sample) were washed three times with PBS buffer (pH=7.4). Then PAAs was added to the treated cells in PBS (pH=7.4), and further incubated for 2 h at 37 °C. The fluorescent signal in living cells could be monitored qualitatively.

5. MTT assay

In order to evaluate the PAAs dose on cellular toxicity, cells treated by the complex with a serial concentration. After treatment, cells were incubated in fresh medium for 24 h. The culture medium was replaced by 100 μL fresh medium containing 0.5 mg/mL 3-[4,5-dimethylthiazol-2-yl]- 2,5-diphenyltetrazolium bromide (MTT) assays reagent, and then incubated at 37 °C and 5% CO2 for 4 h. Then, the MTT containing medium was added with 100 μL of acid/isopropanol solution, in which the concentration of HCl was 0.04 M to dissolve the MTT product, formazan. Viability of non-PAAs-treated control cells was arbitrarily defined as 100%. Finally, the absorption at 490 nm of each well was measured by EL808 ultramicroplate reader. The relative cell viability was recorded and shown (Figure 3).

HeLa cells were cultured in 96-well plates for 24 h and then incubated with different concentration PAAs in DMEM containing FBS (10%) for 12 h at 37°C. After that, the medium was replaced with fresh DMEM, and cells were subsequently irradiated with a DIOMED630PDT-light source at 20 $mWcm^{-2}$ for 30 min. After cultivation for 24 h, the cell viability was detected by using the EL808 ultramicroplate reader. All experiments were done in three times.

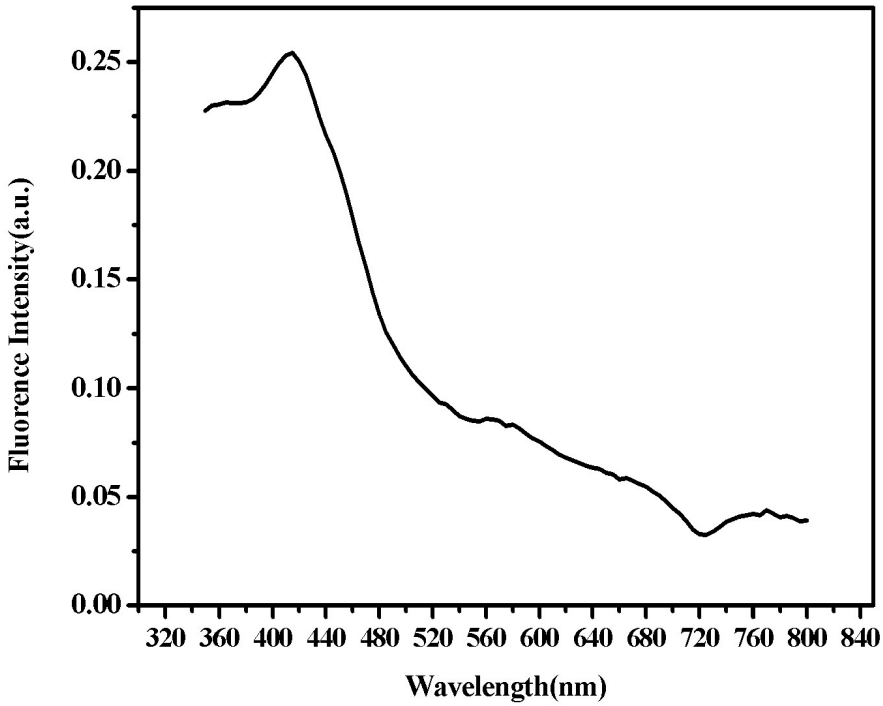

Figure S1.The UV spectrum of SH-AS1411 protected AgNPs

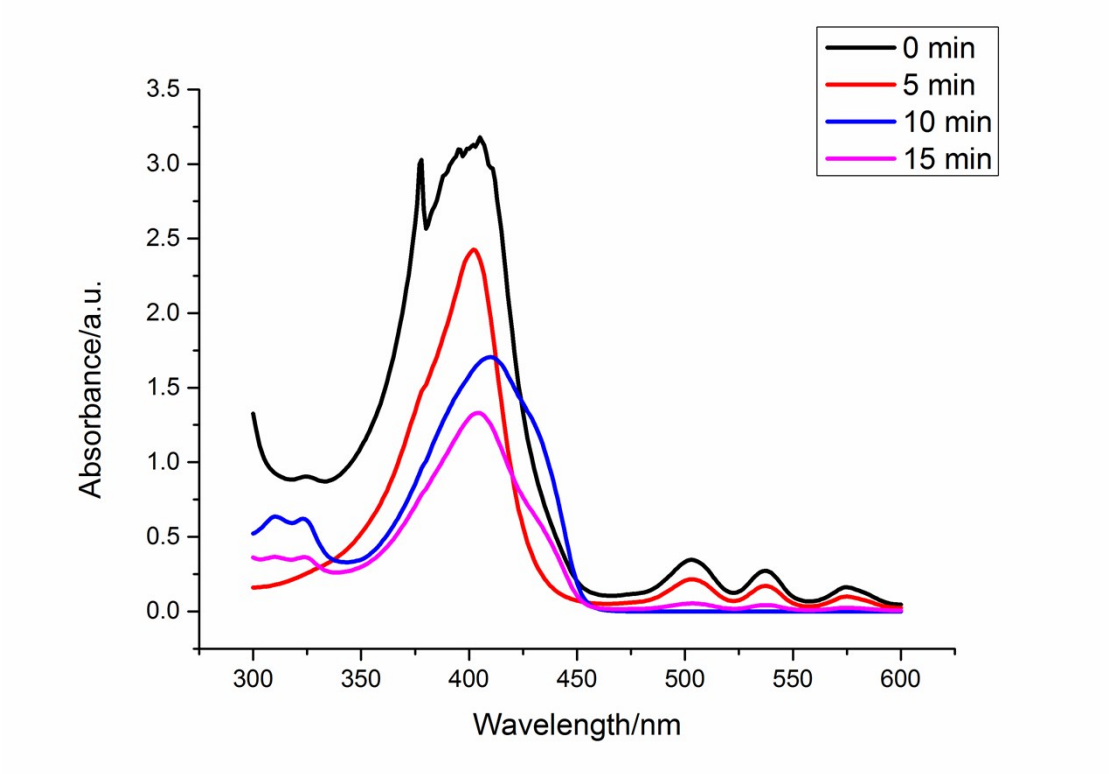

Figure S2. Singlet oxygen characterization. Time-dependent absorption spectra of the 1,3-Diphenylisobenzofuran（DPBF） with PAAs in air.

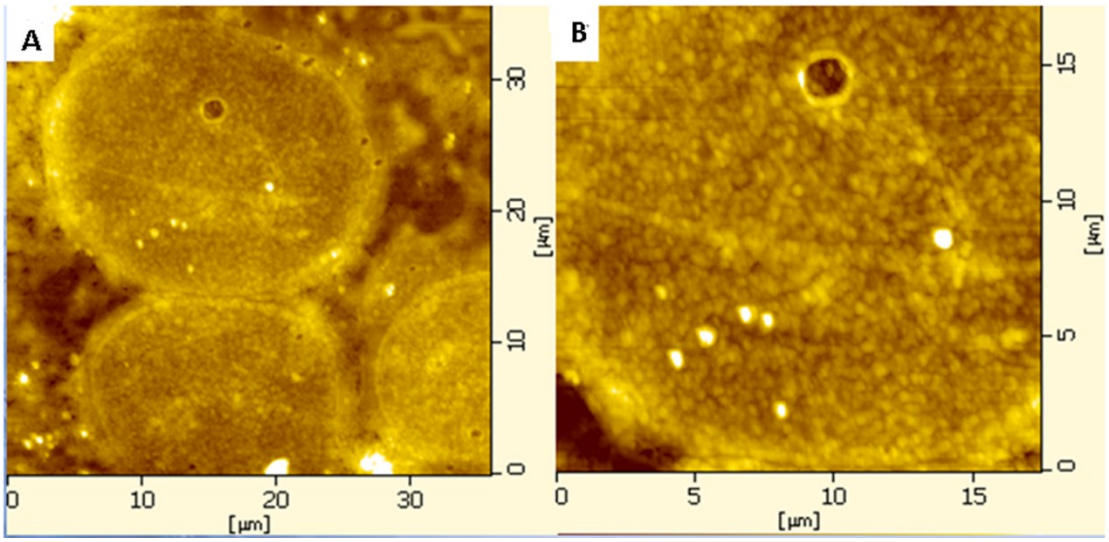

Figure S3 A) AFM image of HeLa cells incubated with PAAs and B) Enlarged AFM image.

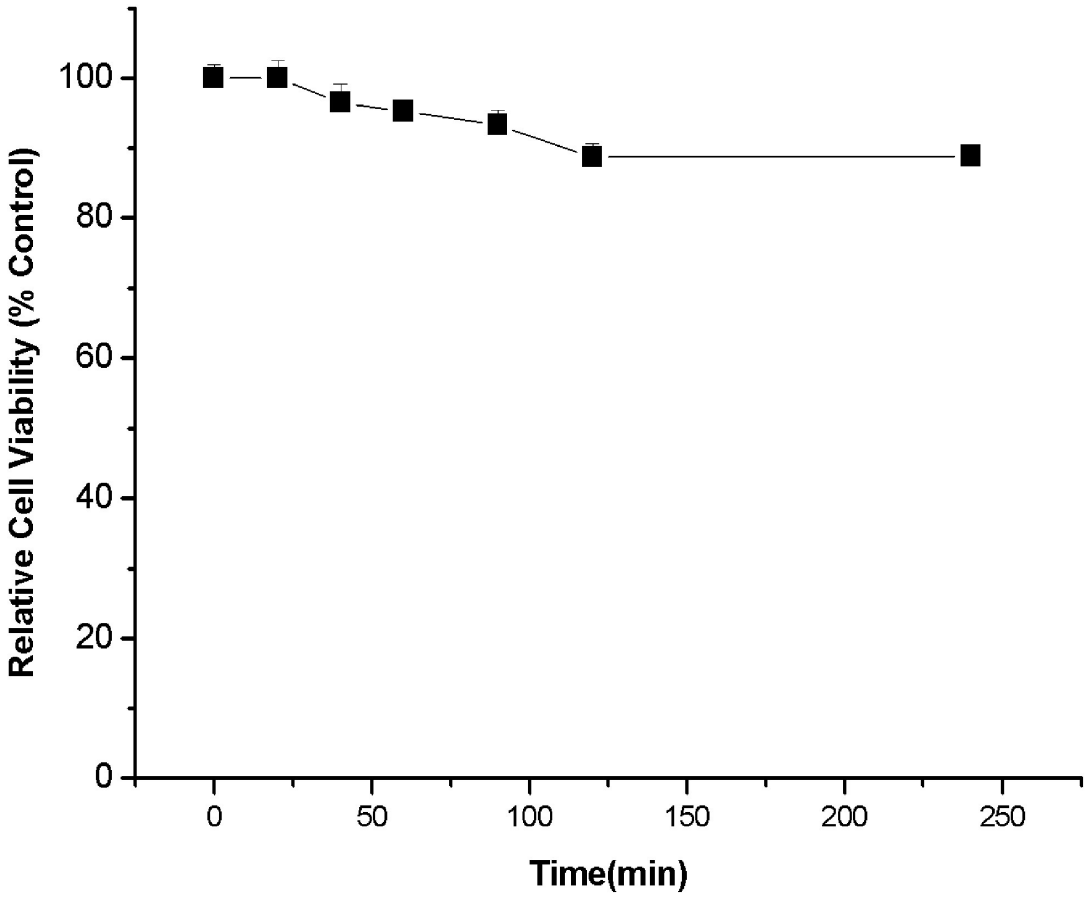

Figure.S4 Optimization of experimental conditions of PAAs by Reaction time from 0 to 240 min.

References

1. W. Dungchai, Y. Sameenoi, O. Chailapakul, J. Volckens, C. S. Henry, *Analyst*. 2013, **138(22),** 6766.

2. J.W. Liu, Y. Lu, *Angew Chem. Int. Ed.* 2006, **45**, 90.