Supporting Information

Dendrimer Porphyrin-coated Gold Nanoshell for Synergistic Combination of Photodynamic and Photothermal Therapy

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Experimental Details

Materials. Dendrimer porphyrin (DP) was synthesized and characterized as described previously.[9] Tetraethyl orthosilicate (TEOS), an ammonia solution, (3-aminopropyl) triethoxysilane (APTES), tetrakis(hydroxymethyl)phosphonium chloride (THPC, 80% solution in water), hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, 99%), hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4{\cdot}3H_2O$), polyehtyleneimine (PEI, 50wt% solution in water, MW 750,000), and 2',7'-dichlorofluorescin diacetate (H_2DCFDA) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). Aqueous ammonia (NH_4OH, Duksan, Korea) and RPMI 1640 (Hyclone, USA) were used without further purification. Phosphate buffered saline (PBS, 0.1 M, pH 7.4) was prepared with 1.1 mM potassium phosphate monobasic, 3 mM sodium phosphate dibasic heptahydrate, and 0.15 M NaCl in deionized (DI) water (Milli-Q Ultrapure, Millipore, Billerica, MA, USA).

Characterization. The particle size was investigated with dynamic light scattering (DLS, Zetasizer nano ZS, Malvern, USA), and the ζ-potentials were monitored using an electrophoretic light scattering spectrophotometer (ELS-8000, Otsuka Electronics Co., Japan). The UV-Vis absorbance spectra were acquired using a UV-Vis spectrophotometer (Optizen 3220 UV, Mecasys Co., Korea). The morphology of the particles was assessed using high resolution transmission electron microscopy (TEM), which was performed with a JEM-3010 microscope at 300 kV (JEOL, Tokyo, Japan). A Zeiss Axiovert 200 microscope equipped with an integrated color CCD camera (Carl Zeiss Inc., Thornwood, NY, USA) was used to obtain fluorescent images. Image analysis was performed using commercially available image analysis software (KS 300, Carl Zeiss Inc.). The temperature of the sample after light irradiation was measured by a thermocouple (TES2732, Multimeter, Type-K) with a minimum detection time of 1 s and a minimum threshold of 0.1 °C.

Preparation of the gold nanoshell-coated silica nanoparticles (AuNS). AuNS, which consist of SNPs coated with a thin layer of gold, were prepared using a method that is similar to a previously described method.[24, 41] Monodisperse SNPs with a mean diameter of 168±3 nm were synthesized using a modified Stöber method, while monodisperse gold nanoparticles (AuNPs) with a mean diameter of 1-2 nm were synthesized by the reduction of chloroauric acid with THPC as a stabilizer. The SNP solution was then centrifuged and washed with ethanol upon ultrasonication to break-off the agglomerates that were formed by the hydrogen bonding between the particles. The AuNP solution was stored in the refrigerator before being used in subsequent steps. The SNPs were functionalized with APTES to facilitate the attachment of the AuNPs. For this approach, 10 mg of SNPs was incubated in 5% v/v APTES in ethanol under vigorous stirring for 2 hours and then gently refluxed for an additional 1 hour to enhance the covalent bonding of APTES to the SNP surface. The excess APTES was removed by washing with ethanol three times using a centrifugation (at 1400 g) / washing protocol. APTES-functionalized SNPs were then resuspended in the gold seed (tiny gold nanoparticles with diameters of 1-2 nm) solution for 20 minutes, followed by three washes. The gold seeds attach to the APTES-functionalized SNPs via a weak covalent bond between the amine functional group of APTES and the surface of the AuNP. The gold seed-attached SNPs were used to generate AuNS. For the growth of the AuNS, a solution of gold hydroxide was prepared. Briefly, 0.05 g of potassium carbonate (K_2CO_3) was dissolved in 185 mL of HPLC-grade water. After 10 minute of stirring, 15 mL of 5 mM $HAuCl_4$ was added to the solution. The transparent mixture initially appeared yellow and slowly became colorless after 1 hour, indicating the formation of gold hydroxide. The resulting solution was aged for 24 hours in the dark before being used in subsequent steps. Next, 1 mg of gold seed-attached SNPs was added to the gold hydroxide solution. Then, 10 mL of a 1.87 mM hydroxylamine hydrochloride solution was added drop by drop, while the change in solution color from purple to blue-green was

monitored. The resultant solution was centrifuged and washed with deionized water upon weak ultrasonication to obtain pure AuNS without large gold nanoparticles. The degree of gold nanoshell coverage could be controlled by varying the amount of gold hydroxide solution used.

Fabrication of DP-coated AuNS (AuNS-DP). The DP-coated AuNS (AuNS-DP) were prepared by a LbL deposition of positively charged PEI and negatively charged DP on the AuNS. Negatively charged AuNS were incubated with 1.0 mg/ml PEI in 0.4 M NaCl (pH 7.4) for 30 minutes. The excess PEI was removed by washing with deionized water three times using a centrifugation (at 1400 g) / washing protocol. Then, the PEI-deposited AuNS were resuspended in 1.0 mg/ml DP in PBS for 30 minutes, followed by three washes, generating the AuNS/DPs. When DP-coated SNPs (SNP-DPs) were prepared, the PEI was deposited on the negatively charged SNPs and the DPs were subsequently immobilized via the same method described above.

In vitro reactive oxygen species (ROS) detection. The intracellular ROS level generated by the DPs after light irradiation was examined by staining with the fluorescent dye H2DCFDA. HeLa cells (5000 cells/well) seeded in 96-well culture plates were treated with 0.2 mg/mL of four different nanoparticles (SNP, AuNS, SNP-DP, AuNS-DP) at 37 °C for 5 hours. Next, the cells were incubated with 50 mL of H2DCFDA (10 mM in DMSO) for 1 hour at 37 °C. Then, the cells were washed with PBS and irradiated with broadband visible light for 15 minutes. Fluorescence images of the intracellular ROS generation detected by H2DCFDA were obtained with a fluorescence microscope, and the fluorescence intensity was quantitatively measured using image analysis software.

Examination of the photothermal effect. To measure the photothermal conversion performance of different nanoparticles, the temperature of solution containing nanoparticles was recorded after irradiation using a 808 nm NIR laser (power density 2 W/cm^2) or broadband visible light from a light-emitting diode (LED; power density 36.67 W/cm^2).

Cytotoxicity assay. HeLa cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics in a humidified atmosphere containing 5% CO_2 at 37 °C. The cells (5000 cells/well) seeded in 96-well culture plates were incubated with various concentrations (200, 100, 50, 25, 12.5, and 6.25 μg/mL) of SNPs decorated with different materials for 1 hour. Careful washing followed to remove the free nanoparticles that were not taken up by the cells, and then, the culture medium was replaced. Photoirradiation was performed for 15 minutes with broadband visible light from a LED or/and was performed for 5 minutes with an 808 nm NIR laser. Meanwhile, the biosafety of the different nanoparticles was investigated using the same method without photoirradiation. Finally, the therapeutic effect and biosafety of various nanoparticles was evaluated with a cell viability assay using 3-(4,5-dimethylthiazchol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).

TEM imaging of the cellular uptake of AuNS-DP. HeLa cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics in a humidified atmosphere containing 5% CO_2 at 37 °C. The cells (5000 cells/well) in 96-well culture plates were incubated with 0.2 mg/ml of AuNS-DP (200 μl) at 37 °C for 1 hour. After washing step, cells were collected and fixed with cold 2% glutaraldehyde in 0.1 M sodium cacodylate buffer at 4 °C for at least 4 hours, followed by aqueous 1% osmium tetroxide and 2% uranyl acetate *en bloc* staining, dehydration in ethanol and finally embedding in Epon/Araldite resin. After polymerizing at 60 °C for 48 hours, sections were cut and imaged after lead and uranyl staining.

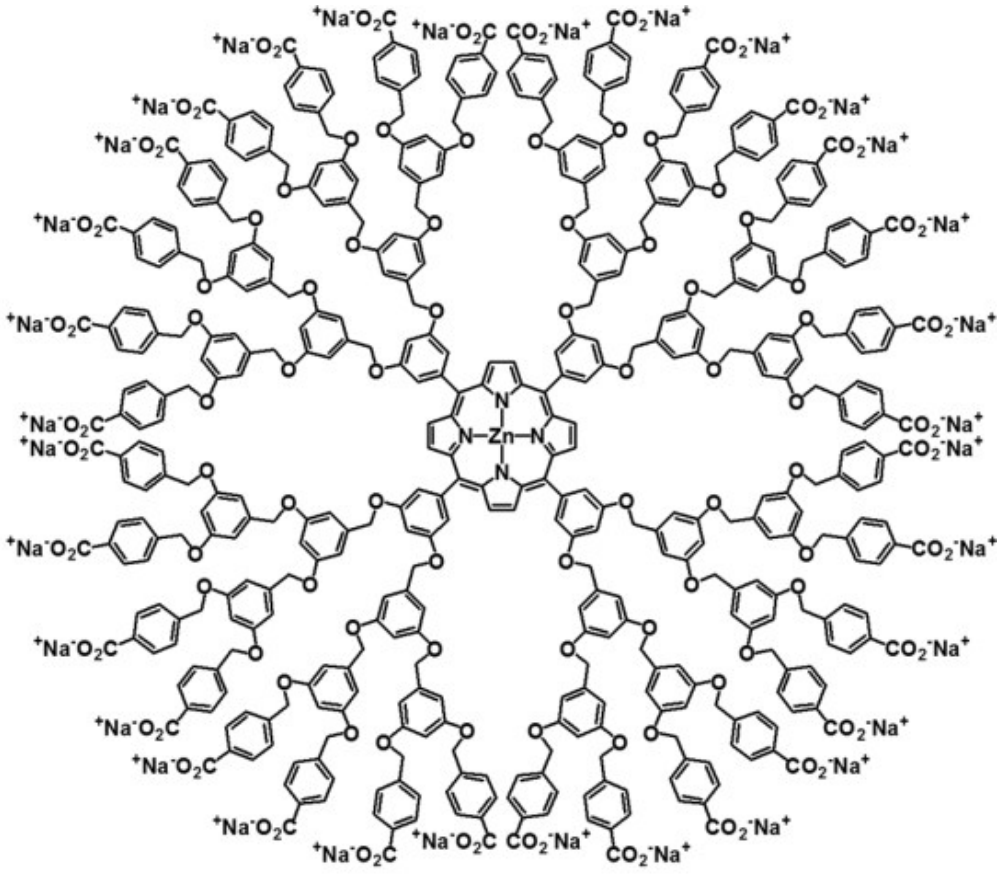


Figure S1. Chemical structure of dendrimer porphyrin (DP).

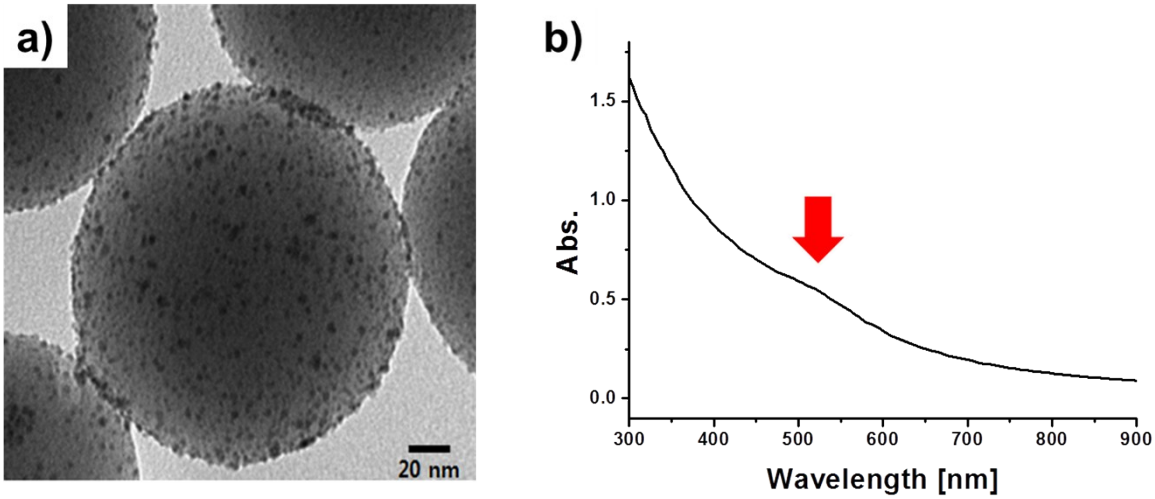


Figure S2. Silica nanoparticles decorated with gold seeds with a 1-2-nm diameter. a) TEM image and b) electronic absorbance spectrum.

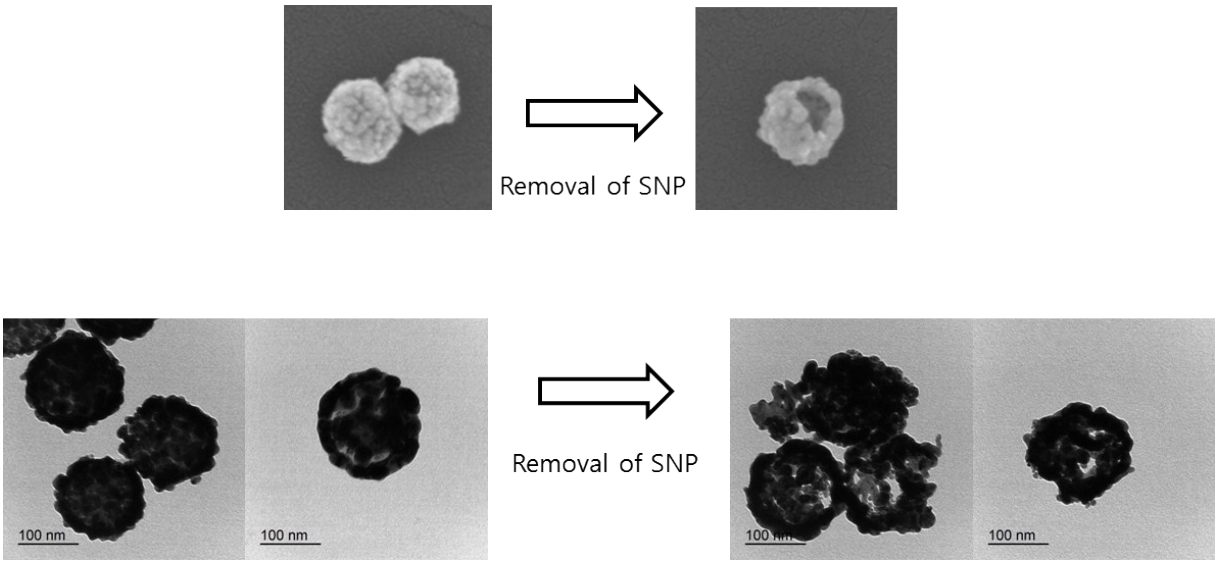
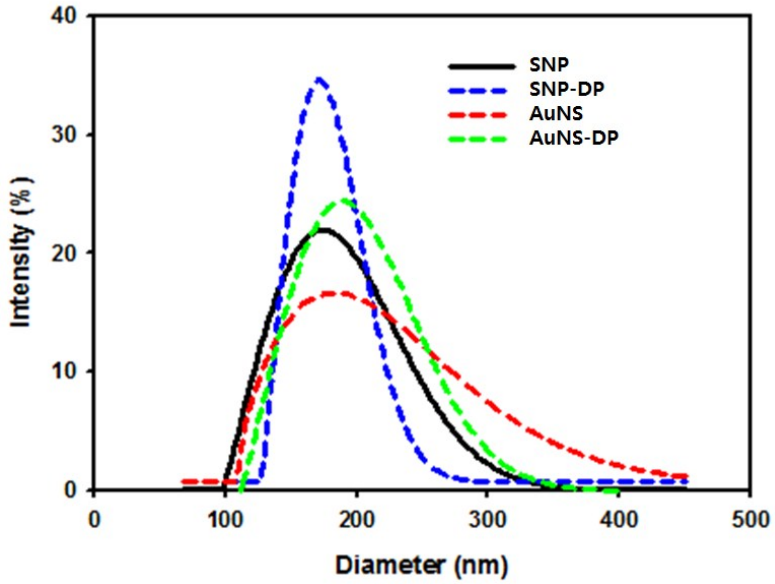
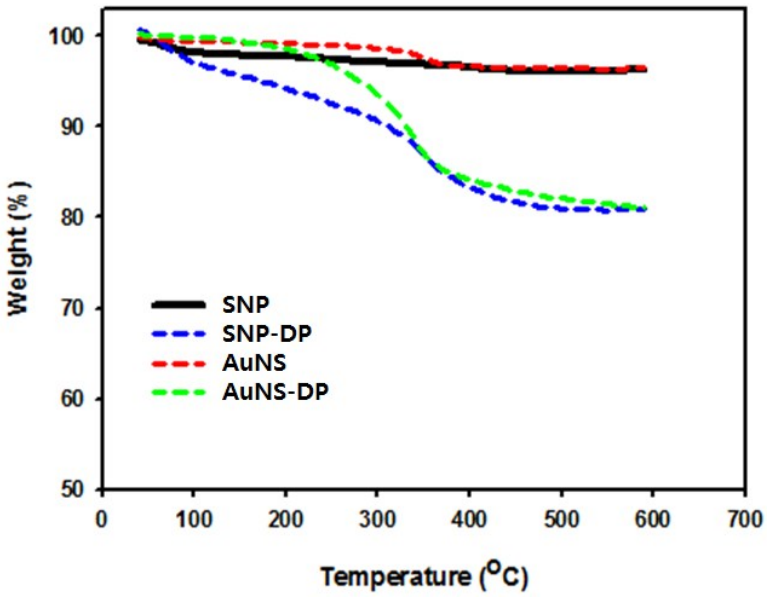


Figure S3. SEM and TEM images of AuNS before and after removal of silica core.



(a)



(b)

Figure S4. Size and thermal analysis of four different nanoparticles (SNP, SNP-DP, AuNS, AuNS-DP). (a) Size distribution profiles obtained by dynamic light scattering (DLS) measurement. (b) Thermogravimetric analysis (TGA) profiles

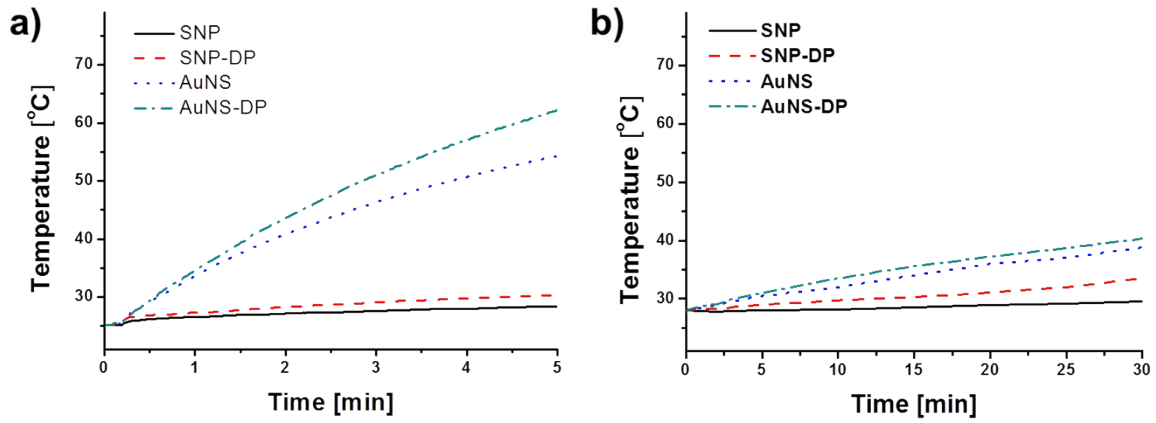


Figure S5. Photothermal effects of nanoparticles with different formulations. Temperature change of the solution containing different nanoparticles as a function of time after a) NIR and (b) visible light exposure.