

Supplementary information

## **DNA templated Synthesis of Branched Gold**

## **Nanostructures with Highly Efficient Near-infrared**

## **Photothermal Therapeutic Effect**

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## Experimental details

**General.** Minimum essential medium with Earle's balanced salts (MEM/EBSS) for cell growth was purchased from HyClone. Fetal bovine serum (FBS) and penicillin–streptomycin (PS) were obtained from GIBCO. Other reagents were obtained from Aldrich, and all chemicals were used as received without further purification. Water was triply distilled using a Millipore filtration system. Solutions of H<sub>2</sub>AuCl<sub>4</sub> and NaBH<sub>4</sub> were freshly prepared in distilled water, and all the reactions were carried out at room temperature. UV/Vis absorption spectra were obtained using an Agilent 8453. Hydrodynamic size measured using Malvern Zetasizer Nano S. Transmission electron microscope (TEM) images were recorded using a JEOL JEM-2100. X-shaped DNA (X-DNA) was fabricated by annealing 4 single-stranded oligonucleotides that were partially complementary to each other. Y-shaped DNA (Y-DNA) was fabricated by 3 single-stranded oligonucleotides.

**Table S1.** Sequence design for X-DNA and Y-DNA building block

Strand	Main body segment
X01	5'-CGA CCG ATG AAT AGC GGT CAG ATC CGT ACC TAC TCG-3'
X02	5'-CGA GTA GGT ACG GAT CTG CGT ATT GCG AAC GAC TCG-3'
X03	5'-CGA GTC GTT CGC AAT ACG GCT GTA CGT ATG GTC TCG-3'
X04	5'-CGA GAC CAT ACG TAC AGC ACC GCT ATT CAT CGG TCG-3'

Strand	Main body segment
Y01	5'-CGA CCG ATG AAT AGC GGT CAG ATC CGT ACC TAC TCG-3'
Y02	5'-CGA GTA GGT ACG GAT CTG CGT ATT GCG AAC GAC TCG-3'
Y03	5'-CGA GTC GTT CGC AAT ACG ACC GCT ATT CAT CGG TCG -3'

**Preparation of the DNA-templated gold nanostructure.** HAuCl<sub>4</sub> dissolved in deionized (D.I.) water was mixed with branched DNA solution in also D.I. water and incubated for 12 hour at 4°C. When mixing the HAuCl<sub>4</sub> with DNA, roughly five equivalent of HAuCl<sub>4</sub> per number of DNA base pair was added. More specifically, 500 uL of 14.7 mM HAuCl<sub>4</sub> was mixed with 100 uL of 200 uM X-DNA (composed of 72 base pair) and 136 uL of 200 uM Y-DNA (composed of 54 base pair), respectively. After incubation, the excess of HAuCl<sub>4</sub> was removed by centrifugation, 14,000 rpm for 50 min, through membrane filter (Microcon centrifugal filter unit, MWCO 3kDa, Millipore). And the left DNA-HAuCl<sub>4</sub> was washed by repeating centrifugation filtering for 5 times against D.I. water. The reduction step to synthesize AuNP was carried by adding 3 equivalent of NaBH<sub>4</sub> per AuCl<sub>4</sub><sup>-</sup> to the solution of DNA-AuCl<sub>4</sub><sup>-</sup> while vigorous stirring at 20°C. After 30 min of reduction, resulting AuNP was washed by repeating centrifugation and resuspension to D.I. water for three times. The sodium citrate was used to improve the particle stability and added after the formation of the branched Au nanoparticles. The morphology and size of nanoparticles were determined by TEM. In brief, a 10 μL aliquot of XGS or YGS in D.I. water was placed onto pre-charged carbon-coated copper grids, air dried and then imaged. The size of the XGS or YGS was determined by analyzing at least 20 particles.

**Ligand exchange of the DNA-templated gold nanostructure.** The XGS and YGS surface was modified using excessive lipoic acid-derived quaternary ammonium ligands and thiolated poly(ethylene glycol) to enhance colloidal stability. The lipoic acid-derived quaternary ammonium ligand was synthesized as described in the previous publication.<sup>1</sup> Excess amount (typically million times excess of the moles of particle) of the surface ligands were dissolved in deionized water. 1 equimolar sodium borohydride was added to the solution and vigorously stirred for 30 minutes at room temperature. The XGS and YGS solution was

mixed to the ligand solution and further stirred for 4 hours at room temperature. To remove excess free surface ligands, the gold solution was centrifuged at 18,000 g, followed by redispersion in distilled water.

**Synthesis of control AuNPs.** An aqueous solution of 99.999% hydrogen tetrachloroaurate hydrate (12.5 ml, 10 mM) was added to 250 ml D.I. water on a stirring hot plate. To the rapidly-stirred boiling solution, 7.5 ml of 50 mM aqueous solution of sodium citrate tribasic dihydrate was quickly added. The color of the solution changed from yellow to purple and finally became red within 5 min. The mixture was removed from heat when the solution had turned deep red and then cooled for 30 min at room temperature. After cooling, the reaction solution was dialysed using Amicon ultra 100 kDa Mw cutoff centrifugal filters for purification. Lipoic acid-derived quaternary ammonium ion (2 ml, 10 mM) and thiolated poly(ethylene glycol) (0.2 ml, 100 mM) solution was mixed to 1 ml of 200 nM AuNP solution and stirred at room temperature. After 10 h, the reaction solution was dialysed three times using Amicon ultra 100 kDa Mw cutoff centrifugal filters for purification.

**Dark-field microscopy.** B16 F10 mouse melanoma cells were purchased from the Korean Cell Line Bank. B16 F10 cells were maintained in MEM/EBSS which was supplemented with 10% FBS and 1% PS. B16 F10 cells were grown onto 12 mm glass coverslips in 24-well plates at a density of  $1 \times 10^5$  cells/well at 37°C under 5% CO<sub>2</sub>. After 1 day, the cells were treated with XGS and YGS. The concentrations were matched for 10 nM XGSs and YGSs when they were co-incubated with cells. These samples were incubated for 6, 24 h at 37°C under 5% CO<sub>2</sub>. The cells were rinsed with PBS three times and fixed with 4% formaldehyde at room temperature for 10 min. The cells were further washed with PBS three times. They were then mounted onto slide glasses using an aqueous mounting medium with an anti-

fading agent (Biomeda). The fluorescence and dark-field images were recorded using a Zeiss Axioplan 2 microscope. A high numerical dark-field condenser (0.75-1.0) and a 100× / 1.3 oil iris objective were used for dark-field images. The pictures were taken using a Zeiss Axiocam HR camera.

**Relative viability levels in dark condition.** B16 F10 cell suspension (5000 cells/well) was dispensed in a 96-well plate and incubated for 1 day at 37°C under 5% CO<sub>2</sub>. Cells were co-incubated in dark condition with the XGS, YGS, and control AuNP. The concentrations of these agents were equivalent to the 10 nM XGS, YGS and control AuNPs. These samples were incubated for 1, 6, 12, 24 h at 37°C under 5% CO<sub>2</sub>. At the end of each incubation time, Cell Counting Kit-8 solution (CCK-8, Dojindo Laboratories) was added to the samples according to the manufacturer's instructions. After further incubation for 2 h, the absorbance at 450 nm was measured using a microplate reader. The results of this measurement are expressed as the ratio between the absorbance of the sample and that of the negative control cells without sample incubations.

**Cancer therapy at the cellular level.** B16 F10 cells were seeded and incubated at 37°C under 5% CO<sub>2</sub> for 1 day. To determine the threshold power densities, cells ( $2 \times 10^5$  cells/well) were grown onto 12-well plates. After 1 day, the cells were co-incubated with XGS or YGS for 12 h. As a control, cells were incubated with the control AuNPs or without any samples. The cells were rinsed with culture medium three times and exposed to laser irradiation for 5 min at different power densities. Trypan blue was applied to the samples at room temperature for 5 min to reveal cell mortality as blue staining.

## Supporting Figures

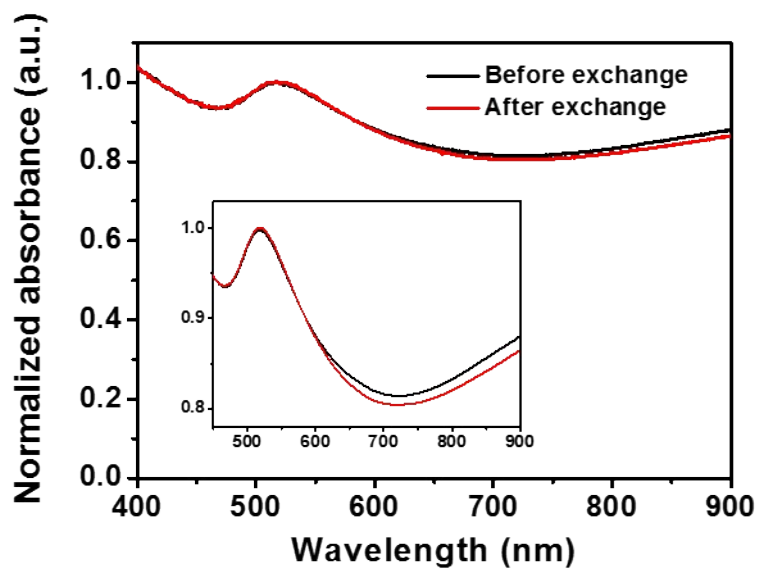


Fig. S1. Before and after ligand exchange, the absorption spectra of XGSs.

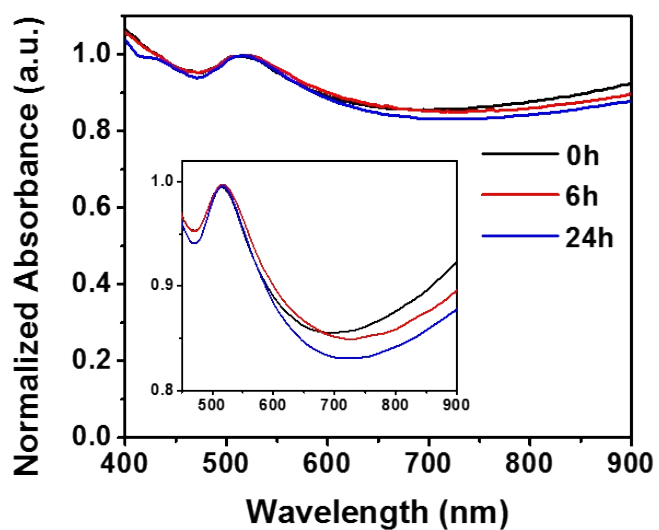
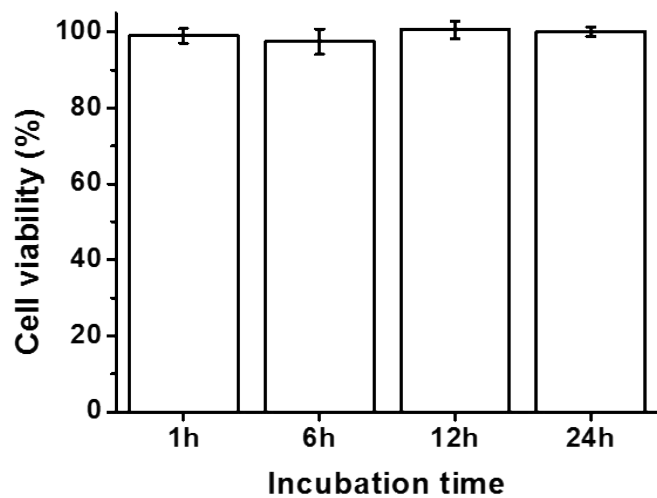


Fig. S2. Absorption spectra of XGSs dispersed in cell culture medium and incubated over 24 h at 37°C under 5% CO<sub>2</sub>.



**Fig. S3.** The cell viability of the B16 F10 cells co-incubated with XGS sample for 1, 6, 12 or 24 h.

#### Reference

- 1 H. Jin, J. Nam, J. Park, S. Jung, K. Im, J. Hur, J.-J. Park, J.-M. Kim and S. Kim, *Chem. Commun.*, 2011, **47**, 1758.