Supplementary information

Title: "Smart" Gold Nanoparticles for Photoacoustic Imaging: Imaging Contrast Agent Responsive to Cancer Microenvironment and Signal Amplification by pH-induced Aggregation

Jaejung Song[a,†] , Jeesu Kim[b,†], Sekyu Hwang[c], Mansik Jeon[d], Sanghwa Jeong[c], Chulhong Kim[b,], and Sungjee Kim[a,c,*]*

[a]*School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology*

[b]*Department of Electrical Engineering and Creative IT Engineering, Pohang University of Science and Technology*

[c]*Department of Chemistry, Pohang University of Science and Technology*

[d]*School of Electronics Engineering College of IT Engineering, Kyungpook National University*

[*]Corresponding authors.

Address: Pohang University of Science and Technology (POSTECH), 77 Cheongam-Ro, Nam-Gu, Pohang, Gyeongbuk 790-784, Republic of Korea

E-mail address: sungjee@postech.ac.kr (S. Kim), chulhong@postech.ac.kr (C. Kim)

[†]These authors contributed equally to this paper.

Materials and Methods

General

Dulbecco's Modified Eagle Medium (DMEM) 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 used as received without further purification. Water was triply distilled using a Millipore filtration system. Solutions of $HAuCl_4$ and $NaBH_4$ were freshly prepared in distilled water, and all the reactions were conducted at room temperature (RT). UV/Vis absorption spectra were obtained using an Agilent 8453 UV-Vis spectrophotometer. Hydrodynamic size measured using a Malvern Zetasizer Nano S. Transmission electron microscope (TEM) images were recorded using a JEOL JEM-2100. PA images were acquired by a custom-designed PA imaging system which consists of a Nd:YAG pump laser (Surelite III-10, Continuum), tunable OPO laser (Surelite OPO PLUS, Continuum), 5-MHz ultrasound (US) transducer (V308, Olympus NDT), amplifier (5072PR, Olympus NDT), and data acquisition system (MSO 5204, Tektronix). Optical and mechanical components of system were purchased from Thorlabs, and assembled manually. To match acoustic impedance, US gel and polyethylene membrane were used.

Synthesis of citrate gold nanoparticles

First, 12.5 mL of 10 mM aqueous solution of hydrogen tetrachloroaurate hydrate (99.99 %) was added to 250 mL deionized water on a stirring hot plate and brought to a rapid boil. Then 7.5 mL of 50 mM aqueous solution of sodium citrate tribasic dehydrate was quickly added. The color of solution changed from yellow to purple and finally to red within 5 min. The mixture was removed from heat when the solution had turned deep red, and was then cooled for 30 min at RT. After cooling, the reaction solution was dialyzed using Amicon ultra 100-kDa-Mw-cutoff centrifugal filters for purification. The morphology and core diameter of

AuNPs were determined by TEM. In brief, a 10 μL aliquot of AuNPs was placed onto precharged carbon-coated copper grids, air dried and then imaged. The core diameter of the AuNPs was determined by averaging measurements from at least 50 particles.

Synthesis of "Smart" gold nanoparticles

SAN were prepared by following the synthesis protocol described in a previous report.[1] The citrate AuNP surface was modified using excessive lipoic acid-derived citraconic anhydride ion ligand. The following is a typical protocol. Briefly, the "smart" ligand (0.13 g, 0.36 mmol) was dissolved in 5 mL water, then an equal molar amount of sodium borohydride (0.029 g, 0.72 mmol) was added to the solution and stirred at RT for 40 min. The solution changed from yellow to transparent as the disulfide group was reduced. The resulting mixture was directly used for surface exchange of citrate AuNP solution (0.72 nmol) without further purification, then stirred at RT. After 10 h, the reaction solution was purified twice by centrifugation using an Amicon ultra 100 kDa-Mw-cutoff centrifugal filter.

Synthesis of Mercaptoundecaonic acid-capped gold nanoparticles

First, 5 mL of citrate gold nanoparticle solution was adjusted to pH 10.5 using 2 M NaOH aqueous solution, then 10 mg of mercaptoundecaonic acid (MUA) was added. The citrate AuNP surface was modified using excessive MUA ligand. The mixture was stirred at RT for 19 h then filtered using a 0.2-μm pore-size syringe filter to remove the excess solid MUA. The reaction solution was dialyzed twice using Amicon ultra 100-kDa-Mw-cutoff centrifugal filters for purification.

Photoacoustic imaging system

To acquire PA images, a custom-designed PA imaging system (Figure S1) was used.[2, 3] Tunable OPO laser was pumped by a Nd:YAG laser with 4-ns pulse width and 10-Hz repetition rate. The wavelength of the output laser be controllable in the range 680-2500 nm. The output laser was delivered by using prisms to a spherical conical lens which generates a donut-shaped laser beam pattern. Diverged beam was focused again at the position of target object by a custom-made optical condenser. A US transducer was posed in the center of condenser to detect PA waves. The detected PA signals were amplified, then saved by the data acquisition system (DAS). To enhance acoustic coupling between the US transducer and target object, custom-designed water tank was used. The bottom of the water tank was opened, then sealed with polyethylene film. US gel was applied between the target object and polyethylene film. The spherical conical lens, optical condenser, and US transducer were aligned and mounted on a rail, and moved together by controlling a scanning stage.

Photoacoustic image reconstruction

Depth-resolved PA signals at each position of the transducer were acquired and saved by the DAS. To acquire a volumetric PA signal, the US transducer and optical beam path were scanned in two axes (i.e., x and y direction) by a motorized scanning system. After the scanning process, PA images of the scanned area were reconstructed by custom-designed Matlab software. The PA image reconstruction was processed in the following procedures: (1) finite impulse response filtering at the center frequency of the US transducer; (2) envelope detection by acquiring the magnitude of a Hilbert-transformed signal; (3) maximum amplitude projection (MAP) to represent volumetric image in the plane; (4) image interpolation to enhance image quality.

Quantification of photoacoustic signals

In each experiment, the amplitudes of PA signals were quantified from the MAP images. Two regions of interest (ROIs) in MAP images were manually selected by the operator. One was the ROI of a signal, and the other was ROI of background. Among the ROIs of the signal, pixels with value less than the mean of ROI of background were excluded during quantification. The remaining pixels in the ROI of the signal were sorted in descending order, and the top 10% were selected. The average and standard deviation of the selected pixels were calculated for quantification.

Photoacoustic signal measurement of "Smart" gold nanoparticles

The PA signals of 10 nM SAN in 20 mM pH 5.5 acetate buffer and 100 mM pH 7.4 PBS buffer were amplified. To contain each sample, silicone tubes (508-001, Dow Corning) were fixed on a custom-designed holder by silicone sealant (RTV18, Momentive). The combined sample holder was posed in the water tank of our custom-designed PA imaging system, and then different-pH solutions were injected at a range of time points (i.e., 0, 5, 10, 15, 30, 60, 90, 120 min) using a 27-gauge syringe. The PA amplitude of each solution was measured at 680 nm. The energy of the laser was 5 mJ/cm^2.

Tissue phantom experiments

HeLa cells and NIH 3T3 cells were incubated in DMEM supplemented with 10 % fetal bovine serum and 1 % penicillin-streptomycin. Cells were grown at 37 °C under 5 % CO_2. After 1 day, SANs were added, then the cells were further co-incubated with SANs for 0, 6, 12, 24, or 48 h. The concentrations of SANs were matched for 100 nM when they were co-incubated with cells. After incubation, the cells were rinsed with culture medium to remove unbound particles.

Cells that contained SAN aggregates were gathered by centrifugation for 3 min at 1500 rpm to remove liquid. Phantoms were fabricated by mixing cell suspension and agar solution (1 % (w/v) agar concentration) at each time point. The mixture of cell suspension and agar solution was used to fabricate tissue phantoms, which were introduced at ~ 10^6 cells/well in each well of a custom-design acrylic plate with ten (5 by 2) 1-cm-diameter cylindrical wells. The agarose gel tissue phantoms containing SANs were embedded in a piece of the well plate, then PA amplitude of the phantom was measured at 680 nm.

To investigate PA signals under various depths of chicken tissue, the phantom was posed under the water tank, and sliced chicken tissues (approximate thickness 5, 10, and 15 mm) were stacked between the phantom and the water tank. The chicken tissues had been were sliced using a razor blade. The thickness of chicken tissue was measured in the PA images. Because this thickness was not uniform, it was calculated by averaging the thickness at five points. The energy of the laser was 5 mJ/cm^2.

Photoacoustic signal measurement in subcutaneous xenograft tumor in mice

Six-week-old female BALB/c nude mice (weights 20-25 g) were purchased from Orient Bio. They were anaesthetized using a vaporized isoflurane gas system (VIP3000, Matrx). HeLa cells (1×10^6) were injected subcutaneously on each flank to generate tumors. The mice were assigned to imaging groups when the tumors reached ~ 5 mm in diameter, as measured with a digital caliper (within 2 weeks of subcutaneous injection of HeLa cells). Then the mice were anaesthetized, and 30 μL of 25 nM SAN or MUA-surfaced AuNP (MAN) solution were injected into their tumors. For measurement of background signal, without injecting SAN or MAN solution, the mice were imaged in grayscale. After injection, the PA amplitude of SAN and MAN was monitored using 680-nm pulsed laser illumination at each time point for 4 h. PA amplitudes of SAN and MAN in normal mice were also monitored. The output energy of

the laser was 5 mJ/cm^2 at 680 nm. All values of PA amplitude were quantified from MAP images. The sizes of the tumors were measured using digital calipers. The standard deviation of the mean calculated from three mice for each sample was used to represent the variability. All experimental animal procedures were approved by the institutional animal care and use committee of the Pohang University of Science and Technology.

Supporting Figures

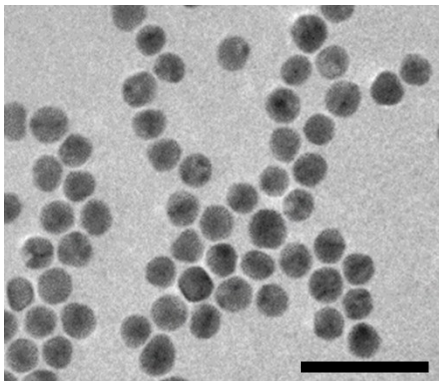

Fig. S1 TEM image of "smart" gold nanoparticles (SANs) (scale bar: 50 nm).

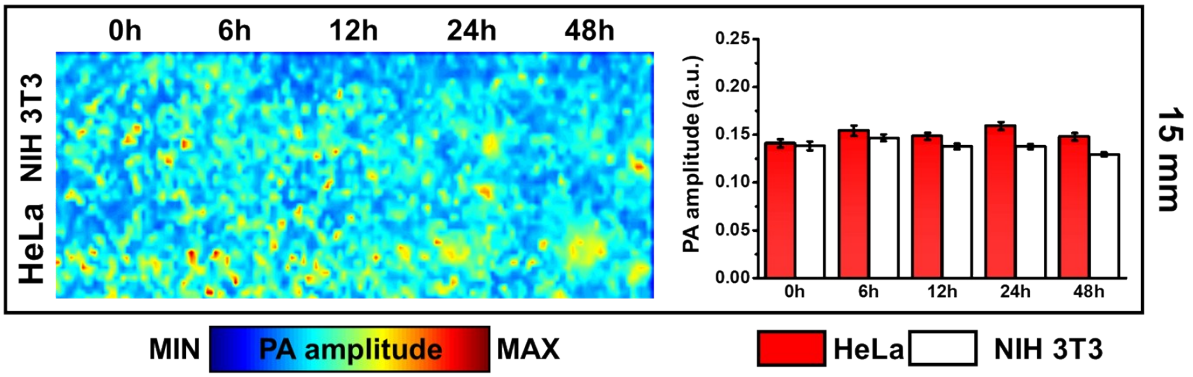

Fig. S2 PA responses of the phantoms consisted with HeLa and NIH 3T3 cells co-incubated with 100 nM SANs in different time at 15 mm thickness of chicken breast tissue.

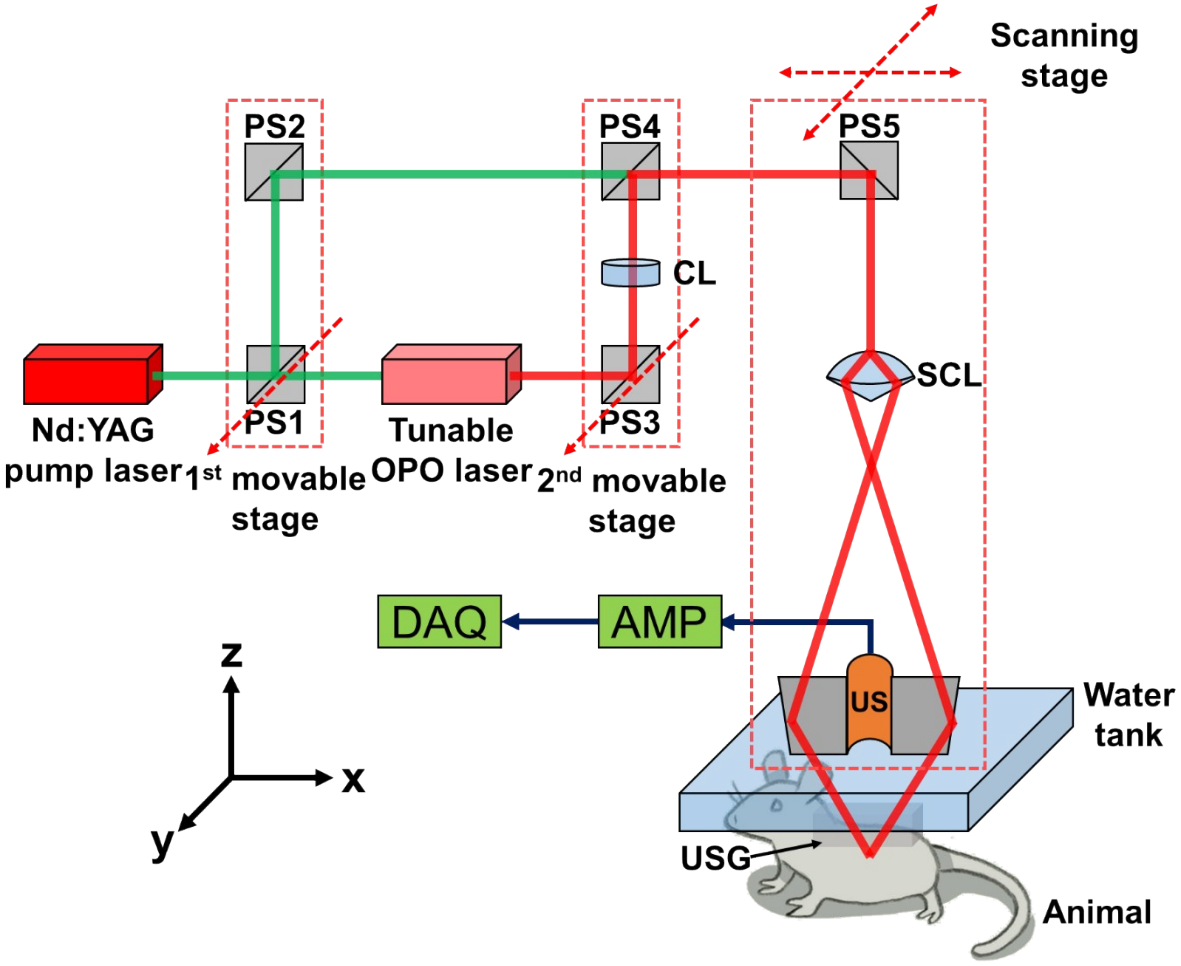

Fig. S3 System configuration of the spectroscopic photoacoustic tomography system. OPO, optical parametric oscillator; PS, prism; CL, collimating lens; SCL, spherical conical lens; US, ultrasound transducer; USG, ultrasound gel; AMP, amplifier; and DAQ, data acquisition.

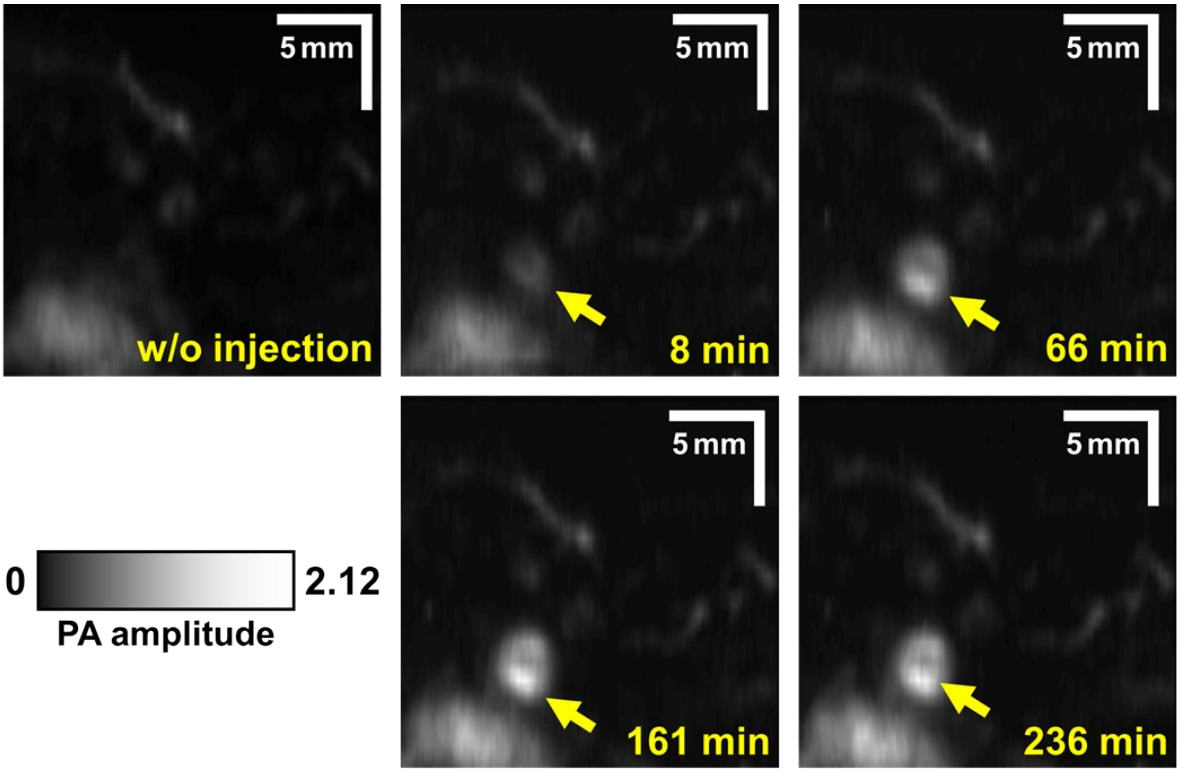

Fig. S4 PA MAP images of the SAN injected tumor *in vivo*. Each image is acquired at before and after injection (8, 66, 161, and 236 minutes). Excitation wavelength of 680 nm is used to monitor PA signal enhancement of SAN. PA, photoacoustic; MAP, maximum amplitude projection; and SAN, "smart" gold nanoparticle.

Reference

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3 C. Kim, M. Jeon and L. Wang, *Opt. Lett.* 2011, **36**, 3599