Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Nitric oxide-activatable gold nanoparticles for specific targeting and photo-thermal ablation of macrophage

Junghong Park,[a,b] Swapan Pramanick,[a,b] Jinhwan Kim,[a,b] Jihyun Lee[a,b] and Won Jong Kim[a,b]

[a] Center for self-assembly and Complexity, Institute for Basic Science, Pohang 37673, Republic of Korea

[b] Department of Chemistry, Pohang University of Science and Technology (POSTECH), Pohang 37673, Republic of Korea

Experimental Section

Materials

N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride(EDC), 8-mercaptooctanoic acid, gold chloride hydrate($HAuCl_4$) N,N-Diisopropylethylamine(DIPEA), *o*-phenylenediamine, N-Hydroxybenzotriazole (HOBt),sodium carbonate, sodium bicarbonate, sodium sulfate, dimethyl sulfoxide-d6 (DMSO-d6), L-arginine, lipopolysaccharide(LPS) and thiazolyl blue tetrazolium bromide (MTT) were purchased from Sigma Aldrich. NO gas was purchased from HANA Gas (Gimhae, South Korea). Peroxynitrite ($OONO^-$) was purchased from Cayman chemical. Griess assay was obtained from Invitrogen. All the solutions were prepared with water purified by a Milli-Q system (Milipore, USA)

Synthesis of NAM.

N-(2-aminophenyl)-8-mercaptooctanamide (NAM) was synthesized by amide coupling using EDC and HOBt as catalyst. Briefly, a solution of 8-mercaptooctanoic acid (180 µL, 1.02 mmol) dissolved in anhydrous methylene chloride (MC) was prepared. EDC (440 mg, 2.30 mmol) and HOBt (380 mg, 2.20 mmol) was added in the solution, and stirred for 10 min. Then, *o*-phenylenediamine (130 mg, 1.20 mmol) with DIPEA (12 mmol) as a base were added in the solution, followed by stirring for 24 h at room temperature. The reaction was checked by thin layer chromatography using 10% methanol in MC. To the reaction mixture, pH 9.2 buffer (0.25 M sodium carbonate & sodium bicarbonate) was added, and extracted with MC for 3 times. The organic layer was dried using anhydrous sodium sulfate. Sticky product with light yellow color product was obtained after evaporation and vacuum oven. *O*-phenylenediamine, 8-mercaptooctanonic acid, and NAM were characterized by ^1H NMR (in DMOS-d6) using 300 MHz NMR spectrometer (Bruker, Germany).

Preparation of NAM-AuNPs.

Citrated capped AuNPs were prepared by following other reports. AuNPs were obtained by reduction of chloroauric acid ($HAuCl_4$) in the presence of citrate ion at high temperature. After $HAuCl_4$ solution (30 ml, 1.47 mM in ultrapure water) was incubated for 15 min at 120 °C, 600 µl of citrate solution (0.34 M in ultrapure water) was added into the $HAuCl_4$ solution. When color of the solution changed from light yellow to ruby red, the solution was stored at 4 °C. To introduce a NO-reacting moiety to AuNPs, citrates were exchanged to NAM through ligand exchange method. NAM (2 mg, 7.51 mmol) was dissolved in 4 ml of 10 % ethanol in water and diluted with water. The diluted solution was added to AuNPs solution (molar ratio of gold : ligand = 1: 34), and shaken mildly for 2 days. Using centrifugal filter tube (MW 3000 Da), remained citrate was removed. The NAM-AuNPs solution was kept under 4 °C. The concentration of AuNPs was determined by Lambert-Beer with known extinction coefficient depending on its size.

Preparation of NO solution.

NO solution was prepared as same method which was already published in other literature. In brief, distilled water (10 mL) was bubbled by 1.36 atm of NO gas for 10 min using NO apparatus which was flushed by argon gas in order to get rid of oxygen. Concentration of NO was determined by Griess assay. Saturated NO solution was found to be 1.88 mM at 20 °C. All of NO stock solutions were freshly prepared before carrying out an experiments.

Quantitative analysis of NO using NAM-AuNPs.

NAM-AuNPs solution (1.7 nM, 400 µL) was mixed with various concentration of NO solution. (100 µL, 3.68, 7.36, 11.04, 14.72, 18.40, 22.08, 25.76, 29.44, 33.12, 36.80, 44.16, and 47.84 µM). After incubation for 1 h, UV-vis absorption spectra from 400 nm ~ 900 nm was monitored by UV-vis spectrophotometer (UV 2550, Shimadzu, Japan) using 1 cm^{-1} path length cells. Then, maximum absorption wavelength (524 nm) was recorded with various concentration of NO.

Characterization of NAM-AuNPs.

TEM (JEM-1011, JEOL, Tokyo, Japan) was utilized to verify distribution and size of NAM-AuNPs. NAM-AuNPs solution (10 µL, 1.36 nM) was dropped into 400 mesh carbon greed (USA, Ted Pella). NAM-AuNPs mixed with NO solution (36.8 µM) for 60 min was also dropped into carbon greed. RAW 264.7 cells were fixed in modified Karnovsky's fixative (2.5 %

glutaraldehyde and 2 % paraformaldehyde in 0.1 mM, cacodylate buffer, with 2.5 mM calcium chloride) at 4 °C for 2-4 h, and washed with distilled water twice at room temperature. The specimen was pre-stained with saturated uranyl acetate at 4 °C for 30 min, followed by dehydration with a series of ethanol solutions of 30, 50, 70, 80,90 and finally with 100 % ethanol three times for 10 min. Then, the sample was rinsed with 100 % propylene oxide twice at room temperature for 15 min, followed by infiltration with spur's plastic. The infiltrated specimen was cut into light-gold-colored ultrathin sections in 90 nm thickness and stained on the grid with uranyl acetate and Reynold's lead citrate. To evaluate the photothermal effect after adding NO, temperature change under NIR irradiation was monitored. For this, 100 µ L of NO solution (0, 3.13, 6.25, 12.5 25, 50 µM) was added in 0.4 mL of NAM-AuNPs (5 nM) solution. After incubated 1 h. the solutions were irradiated by NIR laser (808 nm diode laser, JENOPTIK unique-mode GmbH, Germany) at powder density of 5 W/cm^2.

***In vitro* assay.**

RAW 264.7 cells were cultured in RPMI media containing 10% FBS and 1% penicillin/ streptomycin and incubated at 37 °C in a 5% CO_2 humidified incubator. NIH/3T3 cells were cultured in DMEM media with same condition. Cells were seeded on a 96-well culture plate at an initial density of 8 x 10^3 cells/well and incubated overnight in same condition. Cells were activated by lipopolysaccharide (LPS) for 10 min prior to treatment with NAM-AuNPs (4.65 nM) in RPMI or DMEM media containing 1 mM arginine. After 4 h, the cells were irradiated by 808 nm laser for 20 min. After washing with DPBS, cells were incubated for 20 h. Then, 10 % MTT reagents (5 mg/ml) were added in each well incubated for 4 h. The media was removed and 200 µL DMSO was added to each well to dissolve the purple formazan crystals. Absorption of the solution was measured at 570 nm using microplate spectrofluorometer (VICTOR3 V Mulilabel Counter, Perkin-Elmer-Wellesley, MA, USA). The result was presented as a mean and standard deviation (n=3). For quantitative analysis of NO secreted from RAW 264.7 cell, Griess assay was carried out. Cells (2 x 10^4) were cultured in confocal dish for Live/Dead assay. Live/Dead agents were added in the dish following by manufactory procedure. Images were obtained with fluorescent microscope system (Eclipse Ti-E, Nikon, Kobe, Japan) equipped with CCD camera. (Cool snap MYO, Photometrics, Tucson,AZ)

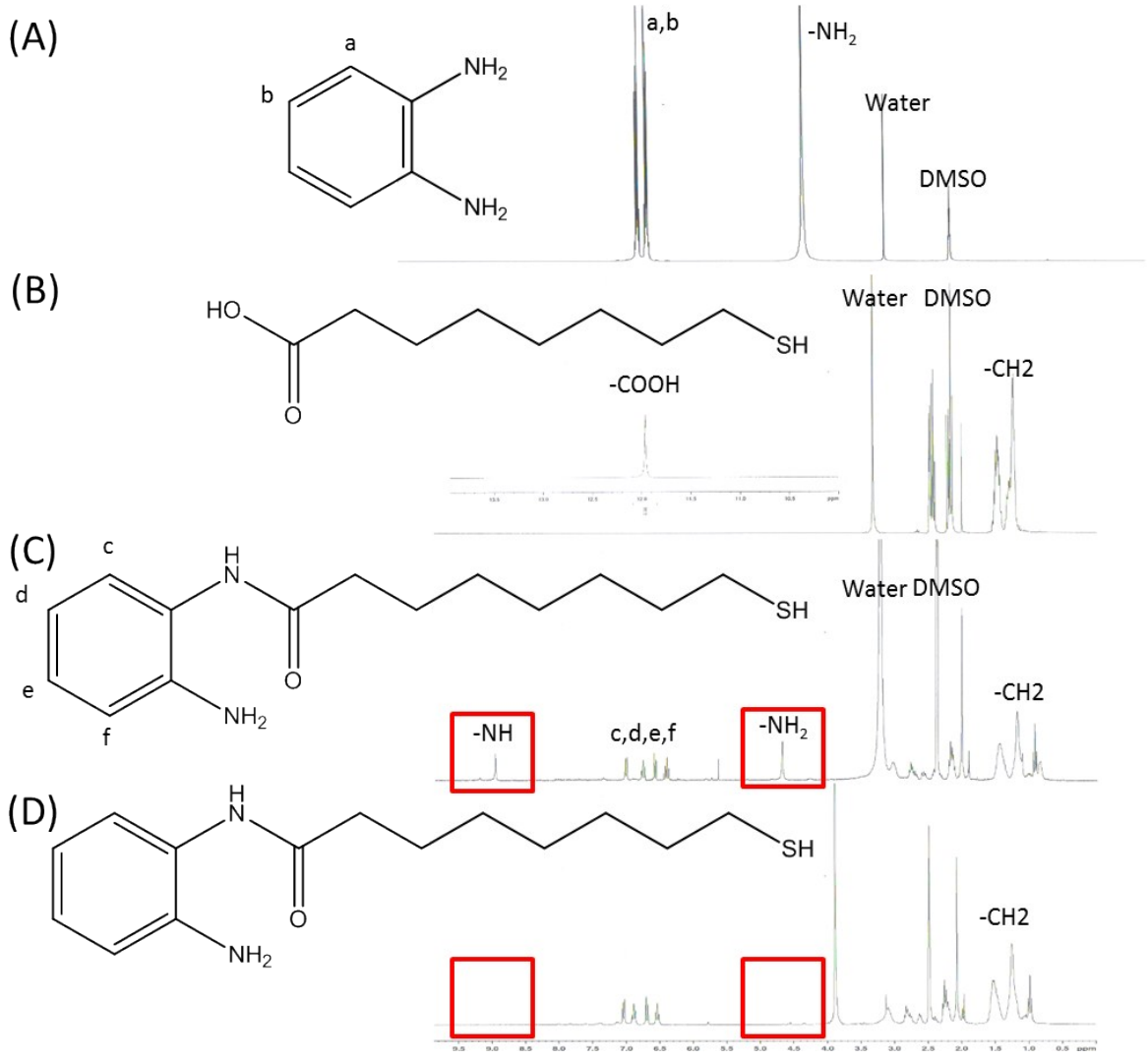

Fig. S1 ^{1}H NMR results. (a) o-phenylendiamine in DMSO-d_6 (b) mercaptooctanoic acid in DMSO-d6 (c) N-(2-aminophenyl)-8-mercaptooctanamide in DMSO-d_6 (d) N-(2-aminophenyl)-8-mercaptooctanamide in DMSO-d_6 after adding a few drops of D_2O and incubated for overnight.

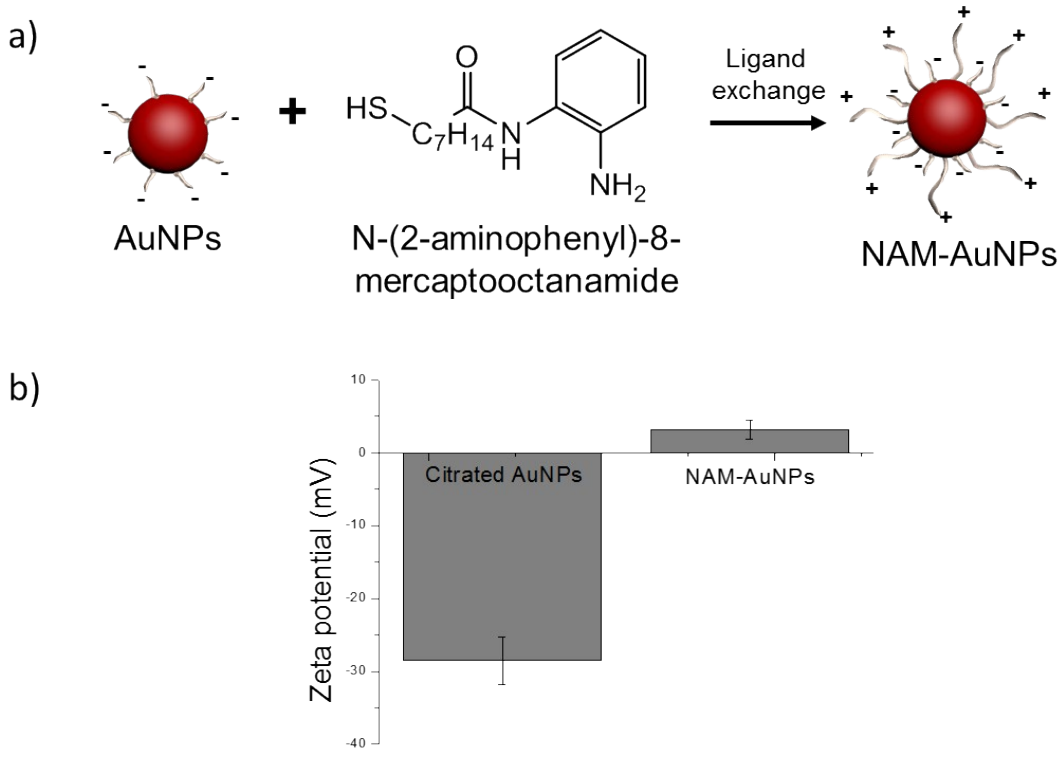

Fig. S2. (a) Preparing the NO-activatable AuNPs (NAM-AuNPs) and zeta potential of gold nanoparticles and (b) zeta potential of NAM-AuNPs.

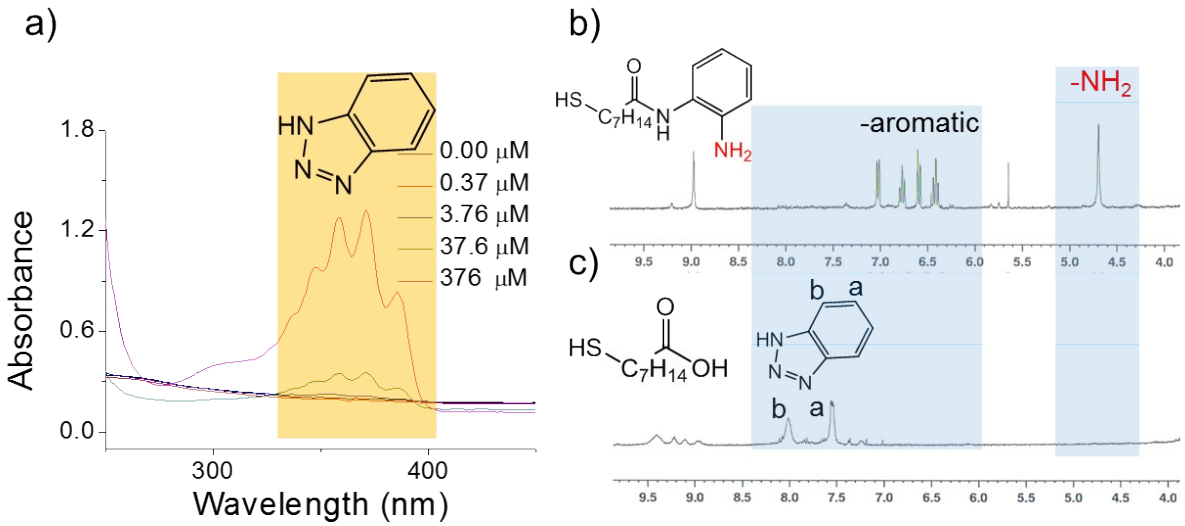

Fig. S3 UV-visible spectrum of NAM-AuNPs after reaction with NO. (a) ^{1}H NMR spectrum of NAM-AuNPs before (b) and after (c) reaction with 100 µM NO for 24 h at 25 ^{o}C

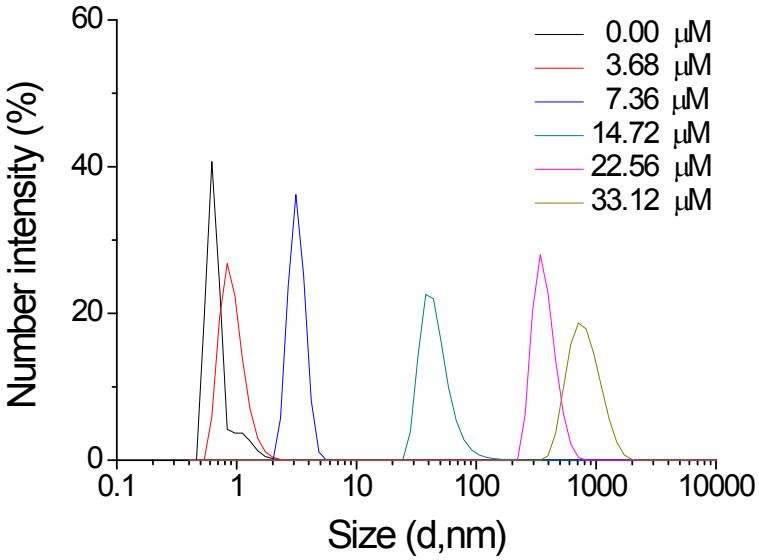

Fig. S4 Size of NAM-AuNPs with NO determined by dynamic light scattering spectrophotometer.

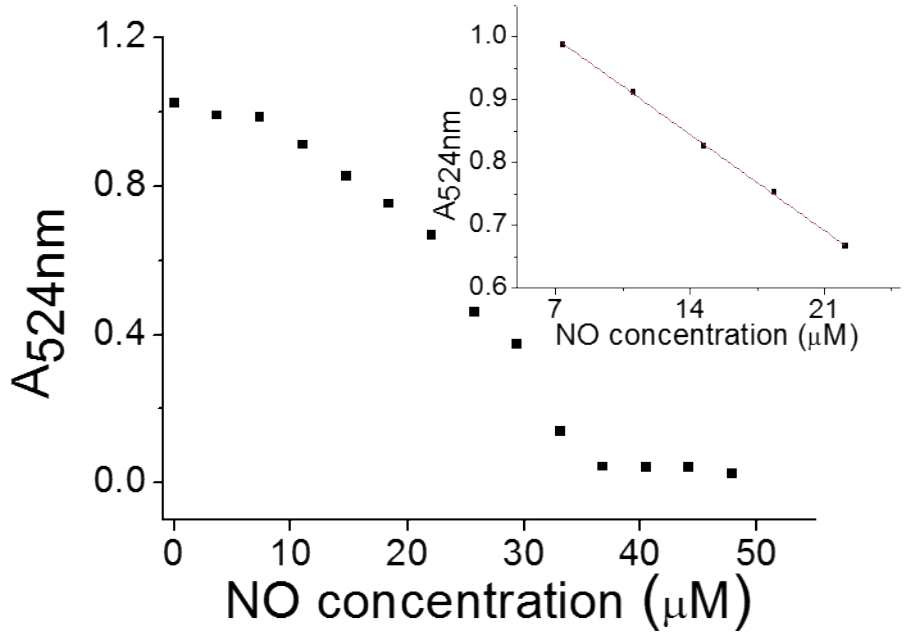

Fig. S5 Absorption of NAM-AuNPs at 524 nm versus the concentration of NO. The inset indicates quantitative linear dynamic range (R^2=0.9992)

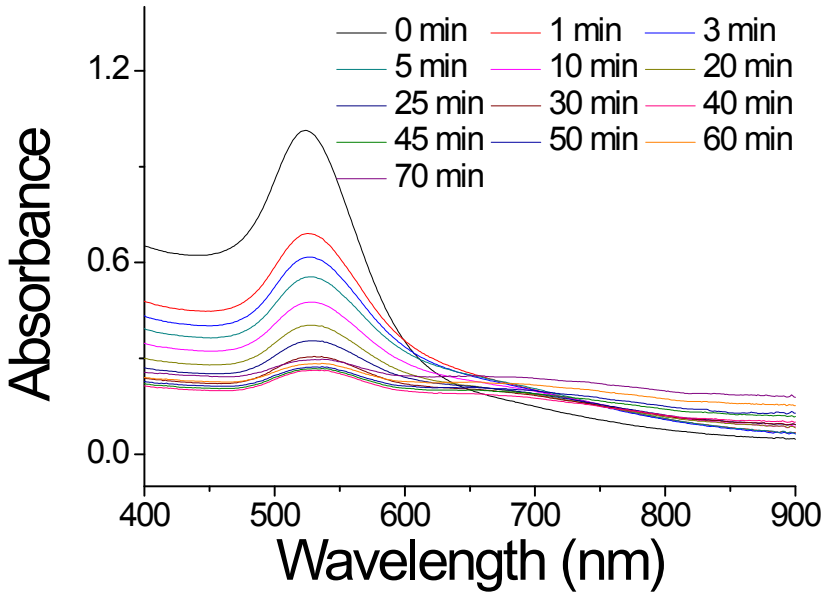

Fig. S6 UV-visible spectrum of NAM-AuNPs solution (1.36 nM) with 36.8 µM of NO at various times.

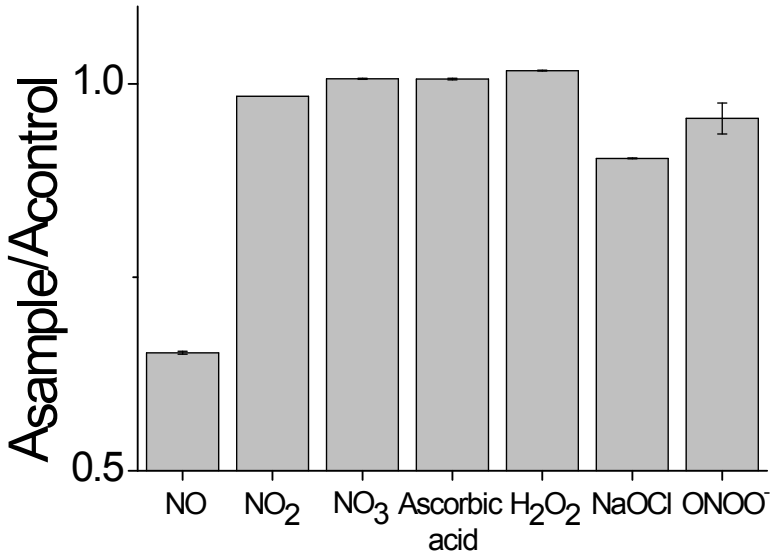

Fig. S7 Selective detection of NO using NAM-AuNPs with 2 mM of reactive nitrogen species, reactive oxygen species and 22.08 μM of NO.

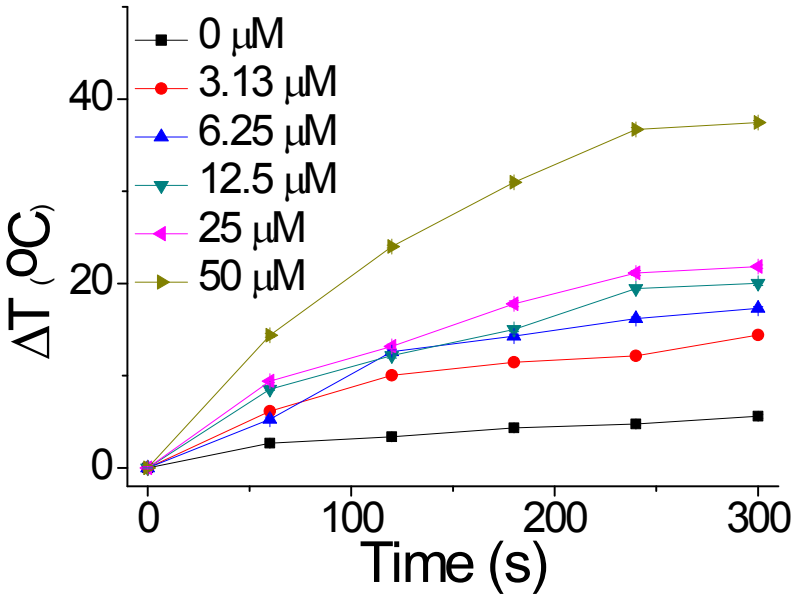

Fig. S8 NIR-responsive temperature change of NAM-AuNPs solution monitored for 5 min after incubation for 60 min in NO solution.

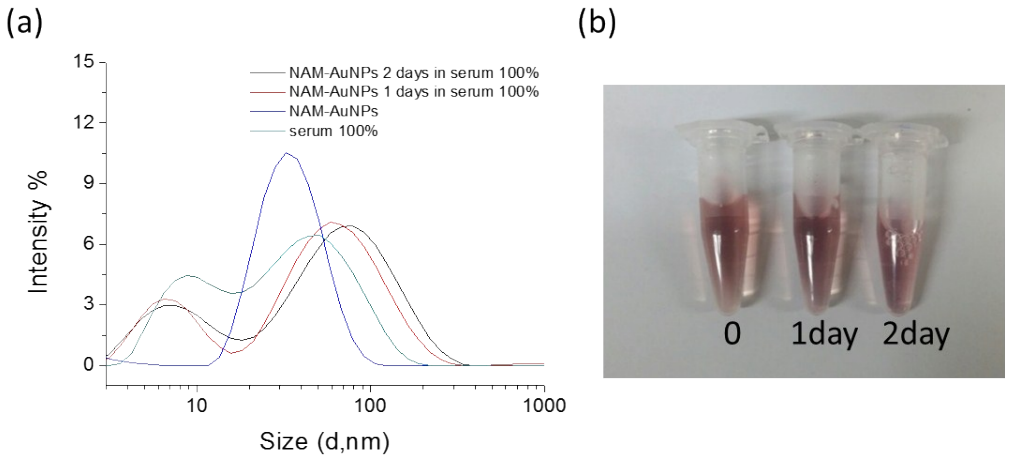

Fig. S9 NAM-AuNPs stability in the serum. (a) DLS DATA OF 100% serum, NAM-AuNPs, NAM-AuNPs in 100% serum in 1 day, 2 day. (b) Real image of NAM-AuNPs with 100% serum for 0 day, 1 day, 2day.