Supporting Information

for

Highly Luminescent Gold Nanocluster Assembly for Bioimaging in Living Organisms

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MATERIALS AND METNODS

Materials

Chloroauric acid hydrate ($HAuCl_4 \cdot 4H_2O$), poly-l-arginine (p-Arg) and sodium hydroxide (NaOH) were purchased from Sinopharm Chemical Reagent Co., Ltd. 6-Aza-2-thiothymine (ATT) was purchased from Alfa Aesar Chemicals Co., Ltd. Glutathione (GSH), L-cysteine (Cys), 3-(4,5-dimethylthiahizol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and N-ethylmaleimide (NEM) were obtained from Sigma-Aldrich. Cell culture media was purchased from Thermal Scientific HyClone. Ultrapure water (18.2 MΩ) was produced by a Heal Force water purifier.

Synthesis of o-Au NCs, g-Au NCs and b-Au NCs

The original Au NCs before PL enhancement were prepared as follows. Orange-emitting glutathione-protected Au NCs (o-Au NCs) were synthesized as follows. 2.5 mM $HAuCl_4$ and 3.75 mM GSH were mixed and reacted at 90 ºC for 18 h under stirring. Green-emitting 6-Aza-2-thiothymine-protected Au NCs (g-Au NCs) were prepared according to Chen et al.'s method with some modifications.[1] Typically, 2.5 mL, 80 mM ATT solution containing 0.2 M NaOH was added into 2.5 mL $HAuCl_4$ (27 mM). Then the mixture was reacted in the dark for 1 h. For preparing blue-emitting cysteine-protected Au NCs (b-Au NCs), NaOH (1 mL, 2 M) was added into Cys (200 mM, 4 mL) aqueous solution first. Then $HAuCl_4$ (0.5 mL, 20 mM) was subsequently added.

The mixed solution was continuously stirred in the dark at 70 ºC for 4 days. All of the new synthesized Au NCs were stored under 4 ºC for further usage.

Fabrication of highly luminescent o-Au NCs/p-Arg, g-Au NCs/p-Arg and b-Au NCs/p-Arg

To realize PL enhancement, p-Arg was introduced to react with the pre-synthesized three kinds of Au NCs and formed o-Au NCs/p-Arg, g-Au NCs/p-Arg and b-Au NCs/p-Arg, respectively. For preparing o-Au NCs/p-Arg, the o-Au NCs were first precipitated out by adding ethanol and re-dispersed in H_2O in the presence of trace of HCl. Then 1.5 mg/mL o-Au NCs was allowed to react with 3 mg/mL p-Arg. To obtain g-Au NCs/p-Arg, the g-Au NCs were dialysed by a 500 Da of dialysis membrane and diluted 2.5 times. Then 1.2 mg/mL g-Au NCs was reacted with 1 mg/mL p-Arg in the dark. For obtaining b-Au NCs/p-Arg, the b-Au NCs were dialysed by a 500 Da of dialysis membrane and diluted 2 times. Then 20 mg/mL b-Au NCs was reacted with 10 mg/mL p-Arg. The reaction condition was optimized by changing p-Arg concentration and reaction pH.

Luminescence analysis of Hg^{2+} and GSH by g-Au NCs/p-Arg

The as-fabricated g-Au NCs/p-Arg were diluted 20 times first and then applied for luminescence analysis of Hg^{2+} and GSH. Hg^{2+} was sensed by virtue of Hg^{2+}-induced PL quenching effect of g-Au NCs/p-Arg. Typically, 500 μL of g-Au NCs/p-Arg diluting solution were added with different concentrations (0-18 μM) of $HgCl_2$ solutions and the gradually decreased PL spectra were recorded. GSH was probed by GSH-induced fluorescence recovery effect of the PL spectra quenched by Hg^{2+}. Different concentrations of GSH (0-24 μM) were respectively added into the g-Au NCs/p-Arg solutions containing 18 μM of $HgCl_2$ and the PL spectra show progressively increased. Specificity of the PL response toward Hg^{2+} and GSH was examined by using various metal ions, salts and amino acids to test the quenching and recovery PL phenomenon.

Cellular Imaging

Before performing cellular imaging experiments, the cytotoxicity of g-Au NCs/p-Arg was examined by standard MTT assays. Briefly, HeLa cells were cultured in

Dulbecco's modified Eagle's medium (DMEM) containing 12% fetal bovine serum, 100 units/mL penicillin, and 100 units/mL streptomycin in 5% CO_2 at 37 °C for 24 h. Then fresh DMEM involving different concentrations of g-Au NCs/p-Arg was applied for further incubating Hela cells. After 24 h, the Hela cells were washed by new-prepared PBS buffer solution for three times and then 20 μL of 5 mg/mL MTT was added for another 4 h incubation. The formed crystalline precipitation was dissolved by 150 μL of DMSO under slow sway for 10 min. The cell viability was evaluated according to the absorbance values at 490 nm measured by the Microplate Reader. For cellular imaging, Hela cells were first incubated in 5% CO_2 at 37 °C for 24 h and then treated with g-Au NCs/p-Arg for 12 h incubation. After washed by PBS buffer solution for three times, the cells were treated by 20 μM of Hg^{2+} for 10 min. In the parallel positive control experiments, exogenous GSH was introduced by adding 50 μM of GSH. In parallel negative control experiments, endogenous GSH was eliminated by 500 μM of NEM.

Zebrafish Culture and Imaging

Zebrafish studies were approved by the Hefei University of Technology Standing Committee on Animals and all experiments were performed in compliance with the relevant laws and international ethics guidelines. Zebrafish were acquired from Nanjing EzeRinka Biotechnology Co., Ltd. and cultured at 28.5 ºC in E3 embryo medium (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 1 mM $CaCl_2$, 0.15 mM KH_2PO_4, 0.05 mM Na_2HPO_4, 0.7 mM $NaHCO_3$, 10^{-5}% methylene blue; pH 7.5). For bioimaging, the embryos or 5-days-old zebrafish were incubated with g-Au NCs/p-Arg in E3 embryo medium for 1 h. After washing with embryo medium to remove the remaining NCs, the embryos or larvae were further treated with 15 μM Hg^{2+} for 10 min. In the parallel positive control experiments, 25 μM GSH were further treated for 1 h. In parallel negative control experiments, 1 mM NEM were added for 20 min to eliminate endogenous GSH. All the fishes were terminally anaesthetized using MS222, and images were collected with fluorescence microscope (Nikon Eclipse Ti2, Japan).

Characterizations

UV−vis absorption spectra and fluorescence spectra were collected by a Shimadzu UV-2600 spectrometer and a F98 fluorospectrophotometer, respectively. Photoluminescence lifetimes were measured by time-correlated single-photon counting (TCSPC) on a Horiba fluoromax spectrofluorometer. Infrared spectra were measured using FT-IR Spectrometer (PerkinElmer Frontier). Transmission electron microscopy (TEM) images were recorded with a JEM-2100F field emission electron microscope at an accelerating voltage of 200 kV. X-ray photoelectron spectra (XPS) were acquired on a Thermo ESCALAB 250 with Al Kα (hν =1486.6 eV) as the excitation source.

References

1. H. H. Deng, X. Q. Shi, F. F. Wang, H. P. Peng, A. L. Liu, X. H. Xia and W. Chen, *Chem. Mater.*, 2017, **29**, 1362-1369.

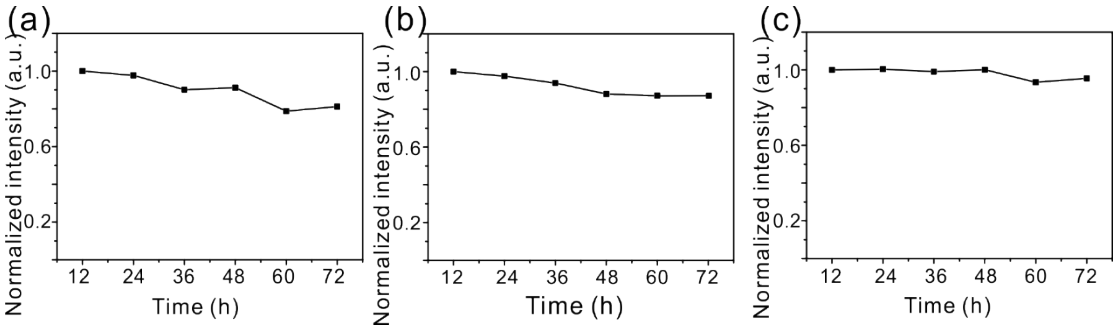

Fig. S1 PL intensity of Au NCs/p-Arg after storing for different times.

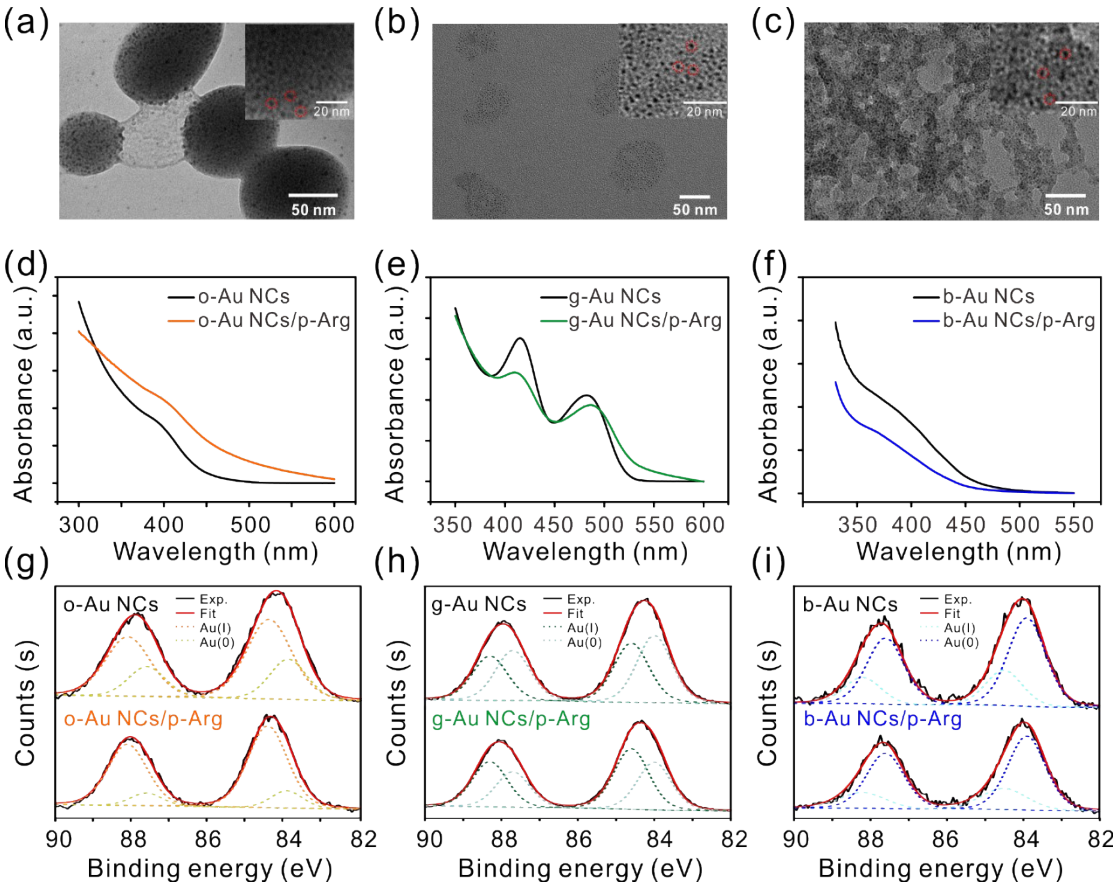

Fig. S2 (a-c) TEM observations on o-Au NCs/p-Arg, g-Au NCs/p-Arg, and b-Au NCs/p-Arg, respectively. (d-f) UV-vis absorption spectra and (g-i) XPS spectra with fitting results of three-color Au NCs before (black lines) and after (colored lines) the p-Arg surface engineering, respectively

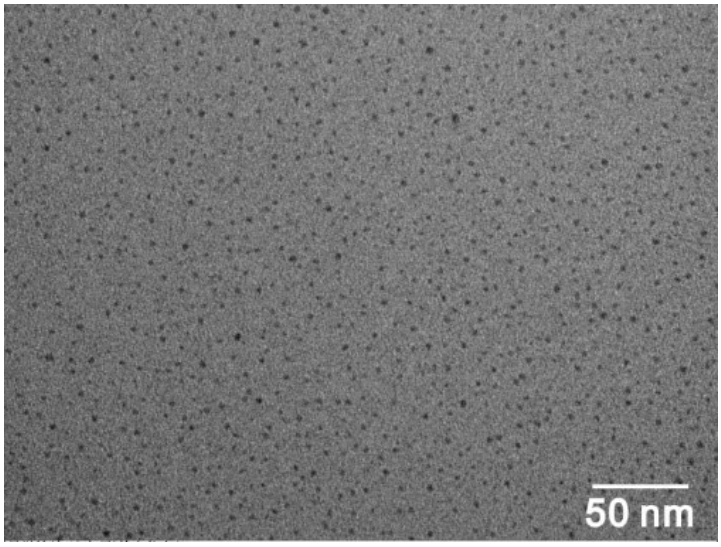

Fig. S3 TEM image of o-Au NCs.

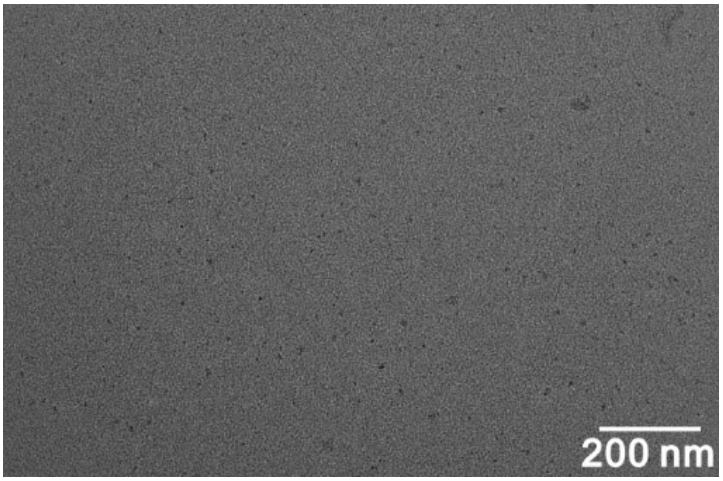

Fig. S4 TEM image of g-Au NCs.

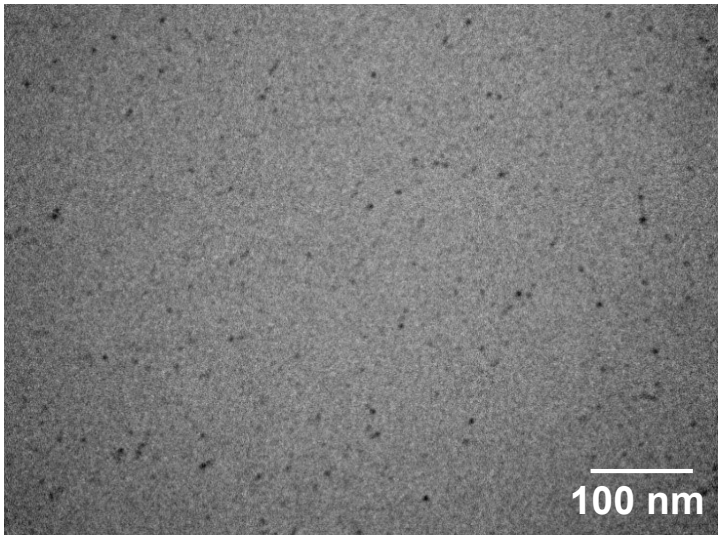

Fig. S5 TEM image of b-Au NCs.

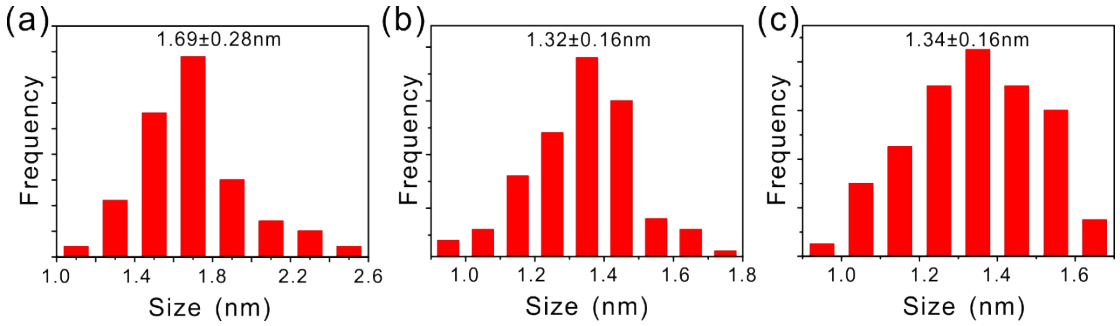

Fig. S6 Size distributions of (a) o-Au NCs/p-Arg, (b) g-Au NCs/p-Arg, and (c) b-Au NCs/p-Arg.

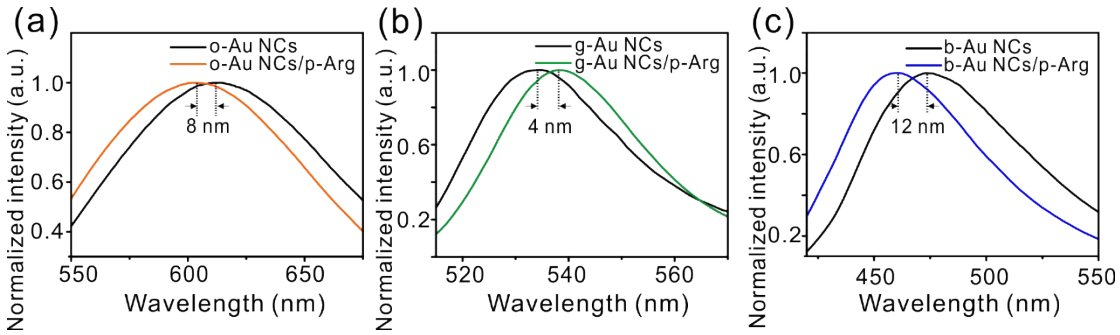

Fig. S7 Normalized PL spectra of three-color Au NCs before (black lines) and after (colored lines) the p-Arg surface engineering, respectively.

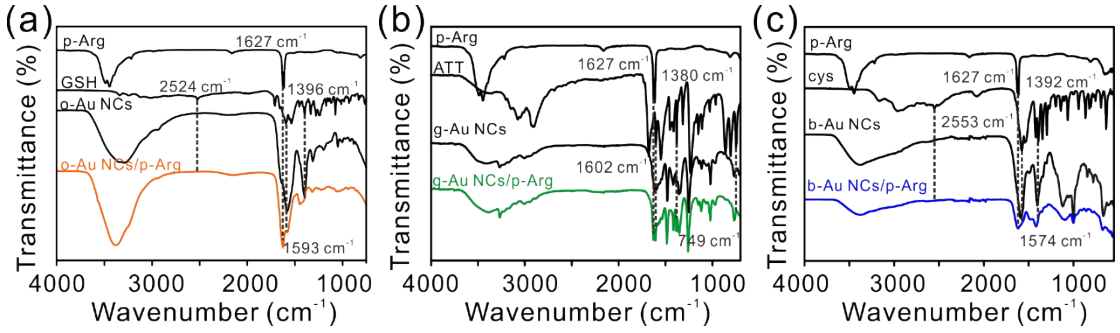

Fig. S8 FTIR spectra of three-color Au NCs before (black lines) and after (colored lines) the p-Arg surface engineering, respectively. The sole GSH, p-Arg and each ligand were used as reference.

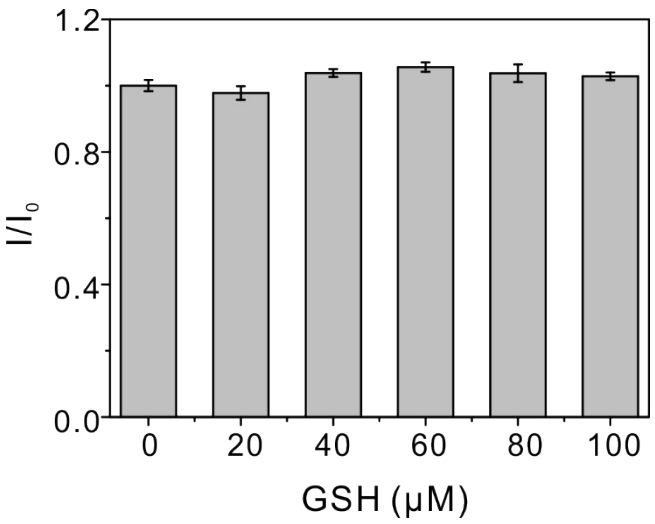

Fig. S9 The effect of GSH with varied concentration on the relative PL intensity (I/I_0) of g-Au NCs/p-Arg.

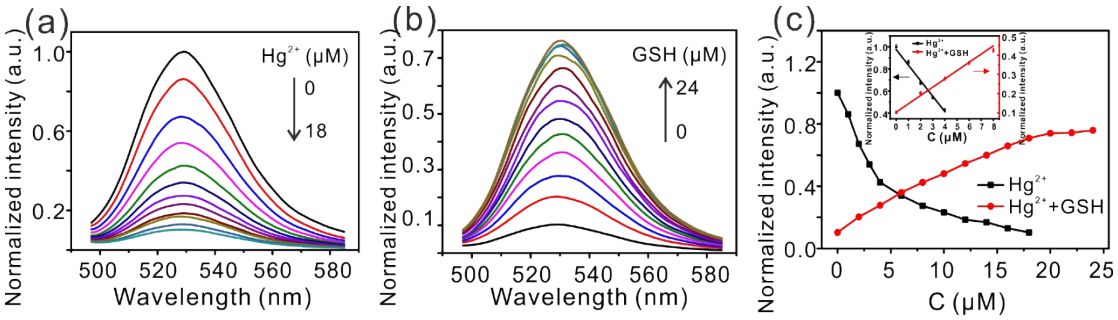

Fig. S10 (a) Normalized PL spectra of g-Au NCs/p-Arg upon addition of Hg^{2+} with concentrations from 0 to 18×10^{-6} M. (b) Normalized PL spectra of Hg^{2+}-quenched g-Au NCs/p-Arg upon addition of GSH with concentrations from 0 to 24×10^{-6} M. (c) Normalized PL intensity plots for g-Au NCs/p-Arg vs Hg^{2+} concentration and Hg^{2+}-quenched g-Au NCs/p-Arg vs GSH concentration. The inset showed the linear fitting in the region of 0-4×10^{-6} M Hg^{2+} and 0-8×10^{-6} M GSH, respectively.

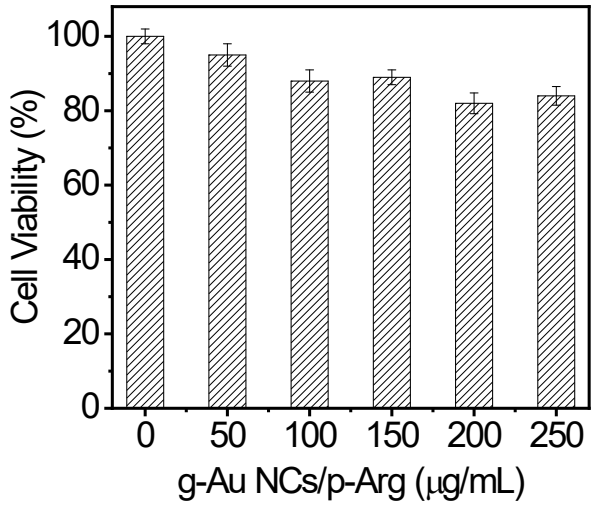

Fig. S11 MTT assay of HeLa cells treated with different concentrations of g-Au NCs/p-Arg for 24 h.

Table S1. Summary of lifetimes of Au NCs and p-Arg-modified Au NCs with the multi-exponential fitting parameters.

Sample	τ1	τ2	τ3	τ4	$\tau_{Average}$
o-Au NCs	6.55 μs (12%)	2.72 μs (39%)	0.86 μs (49%)		2.27 μs
o-AuNCs/p-Arg	1.56 μs (29%)	5.76 μs (49%)	11.06 μs (22%)		5.71 μs
g-Au NCs	1.77 ns (22%)	7.47 ns (6%)	0.28 ns (72%)		1.04 ns
g-AuNCs/p-Arg	4.48 ns (55%)	13.34 ns (20%)	0.91 ns (25%)		5.36 ns
b-Au NCs	0.61 ns (24%)	2.23 ns (13%)	5.6 ns (3%)	0.11 ns (60%)	0.67 ns
b-AuNCs/p-Arg	0.66 ns (30%)	2.63 ns (17%)	5.68 ns (5%)	0.11 ns (48%)	0.98 ns