Electronic Supplementary Information *for*

Viscosity-Sensitive Thiolated Gold Nanoclusters with Diffusion-Controlled Emission for Intracellular Viscosity Imaging †

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1. Experimental Details.

Materials and Reagents. Triple-distilled water was utilized throughout the whole experimental process. Hydrogen tetrachloroaurate trihydrate ($HAuCl_4 \cdot 3H_2O$) was obtained from Aladdin Company (Shanghai, China), and L-glutathione in the reduced form (GSH) and nystatin was purchased from J&K Scientific Company (Shanghai, China). Human A549 cells were bought from Nanjing KeyGEN Biotech. Co. Ltd. HEPES buffer solution (10 mM, pH 7.0) was prepared by mixing stock solution and adjusted to pH 7.0. All reagents were of analytical grade.

Synthesis of Glutathione-Protected Gold Nanoclusters (GSH-AuNCs). In a 20 mL three-necked flask with 4.5 mL of ultrapure water, a certain amount of $HAuCl_4$ (20 mM, 0.50 mL) and glutathione (100 mM, 0.15 mL) were added into the above three-necked flask at room temperature. The reaction mixture was heated to 70 °C with gentle stirring (500 rpm) and the solution became clear after 10 minutes. Then the reaction was continued under nitrogen for 24 hours to form an aqueous solution of GSH-AuNCs. Finally, it was purified by a dialysis bag (1000 Da) to remove excess glutathione. After purification, the orange-emitting GSH-AuNCs solid was collected for the following use.

Solvent and pH-Controlled Aggregation-Induced Emission of GSH-AuNCs. Poor solvent-induced AIE was performed as follows. A given amount of GSH-AuNCs solution (50 μL, 9.0 mg/mL) was added respectively to a series of ethanol/water solutions (3 mL), and the volume ratios of ethanol in the mixed solutions were 0%, 15%, 30%, 45%, 60%, 75%, 90% and 100%, respectively. The concentration of GSH-AuNCs in each solution was 1.5 mg/mL. The resulting AuNCs solutions were monitored at the excitation of 350 nm with an Edinburgh FLS 980 spectrometer. The pH-controlled aggregation-induced emission of AuNCs was conducted as follows. A certian amount of GSH-AuNCs solution (50 μL, 9.0 mg/mL) was added respectively to a series of aqueous solutions at different pHs from 2.0 to 7.0. The concentration of AuNCs in each solution was 1.5 mg/mL. The

PL spectra of the resulting solutions were recorded with an Edinburgh FLS 980 spectrometer.

The Diffusion-Controlled Emission of AuNCs Regulated by Viscosity and Temperature. A series of visocous solutions were first prepared by mixing water and glycerol with different glycerol/water ratios (0%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% and 100%,). At room temperature, the corresponding viscosity values were measured by a digital display viscometer (NDJ-5S model), which were determined to be 10 mPa.s, 17 mPa.s, 24 mPa.s, 26 mPa.s, 27 mPa.s, 37 mPa.s, 50 mPa.s, 66 mPa.s, 104 mPa.s, 336 mPa.s and 991 mPa.s. respectively. A viscous solution (3.0 mL) was first placed in a cuvettes, and then a certain amount of GSH-AuNCs solution (50 μL, 9.0 mg/mL) was added dropwise into the above cuvette. The resulting solutions were recorded with an Edinburgh FLS 980 spectrometer under the same detection conditions. The temperature-dependent luminescence experiment was carried out using a HEPES buffer solution (pH 7.0) of AuNCs (1.5 mg/mL). The PL spectra of AuNCs were measured in the range of 0 – 70 °C at intervals of 5 °C.

Cells Viability Test of GSH-AuNCs and Confocal Microscopy Imaging for Viscosity in Live Cells. Human A549 cells were used to assess the effect of AuNCs on cell viability using a standard MTT assay. In a typical procedure, human A549 cells were seeded in 96-well U-shaped plates at a density of 6000 cells per well, and then incubated with GSH-AuNCs at different concentrations (1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 g/L, respectively) at 37 °C for 24 hours. After discarding the medium, a certain amount of MTT solution (0.1 mL, 0.5 mg/mL DMEM) was added to each well, and the incubation was continued at 37 °C for 4 hours. The supernatant was then discarded and a volume of DMSO (110 μL) was added to each well to dissolve the formed wax. The absorbance value was recorded using a microplate reader at 490 nm in a sterile environment at 37 °C for 10 minutes. Cell viability (V_R) was calculated according to the following formula: $V_R(\%) = A/A_0 \times 100\%$, where V_R is cell viability, A is the absorbance

measured for cells incubated with AuNCs, and A_0 is the absorbance of the control group.

In the imaging experiment, four groups of human A549 cells were treated at different conditions. The first group as the control was performed as follows. Human A549 cells were incubated in DMEM medium for 12 hours. The second group was first incubated in DMEM medium for 12 hours, and then incubated with only GSH-AuNCs (10 μg/ml) for 30 minutes. The third group was first incubated with a certain amount of nystatin (10 μM) for 45 min, and then treated with AuNCs (10 μg/ml) for 30 min. The fourth group was first incubated with a relatively high concentration of nystatin (20 μM) for 45 min, and then treated with AuNCs (10 μg/ml) for 30 min. Prior to imaging using a laser confocal fluorescence microscopy, all three groups of cells were first washed three times with phosphate buffered saline (PBS) to remove excessive GSH-AuNCs. Fluorescence imaging experiments were performed at 37 °C at the excitation of 405 nm, and emissions were collected at a range of 500-700 nm.

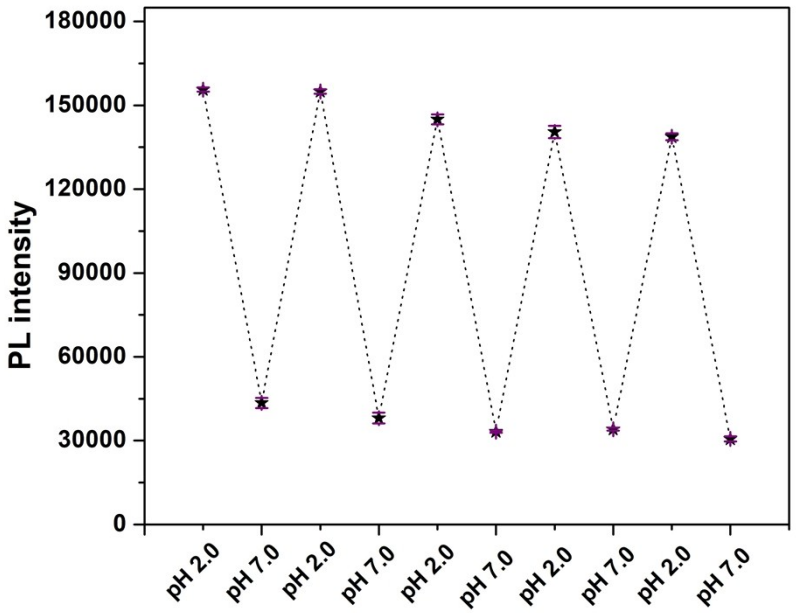

Figure S1. The reversibility test of pH-controlled aggregation-induced emission of AuNCs.

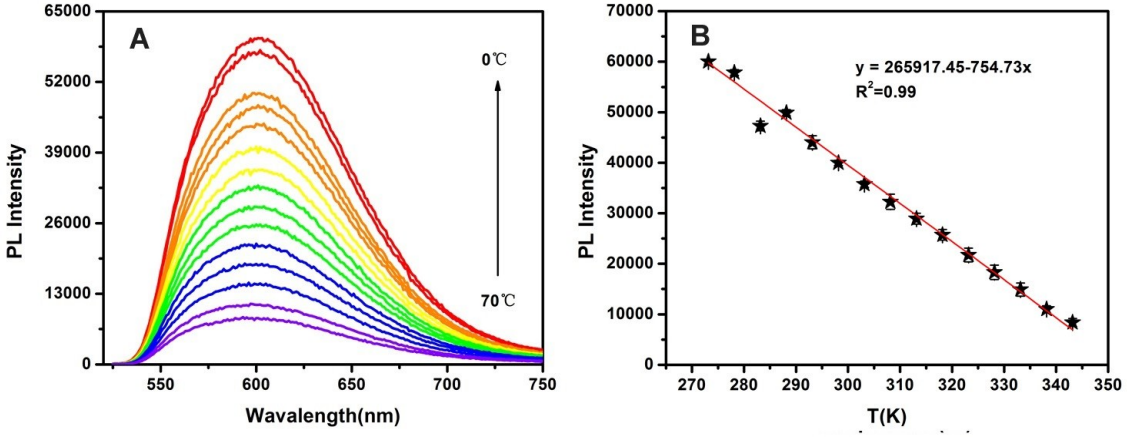

Figure S2. (A) Luminescence intensity of AuNCs in aqueous solution at different temperatures ranging from 0 – 70 °C. (B) Luminescence intensity I versus temperature in the range of 273 – 343 K.

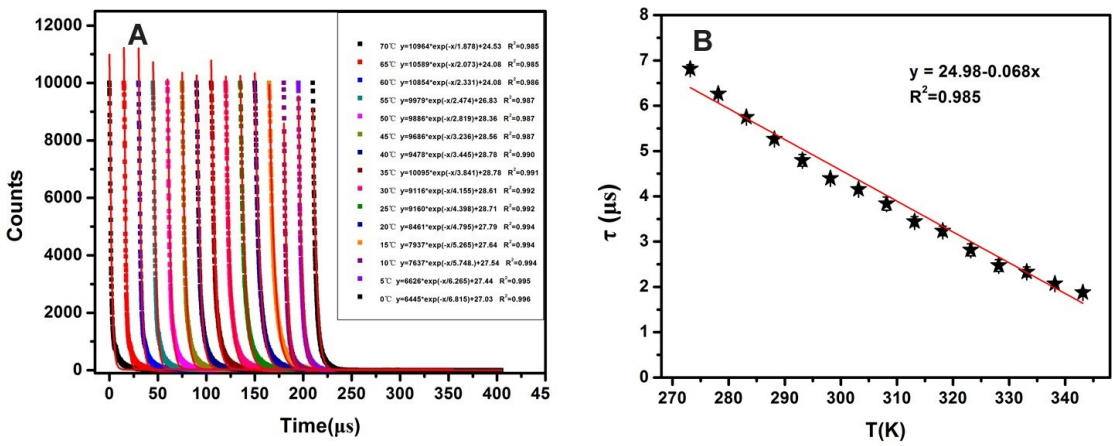

Figure S3. (A) Time-resolved luminescence decay curves of AuNCs at 605 nm at different temperatures. (B) Luminescence lifetime τ versus temperature in the range of 273 – 343 K.

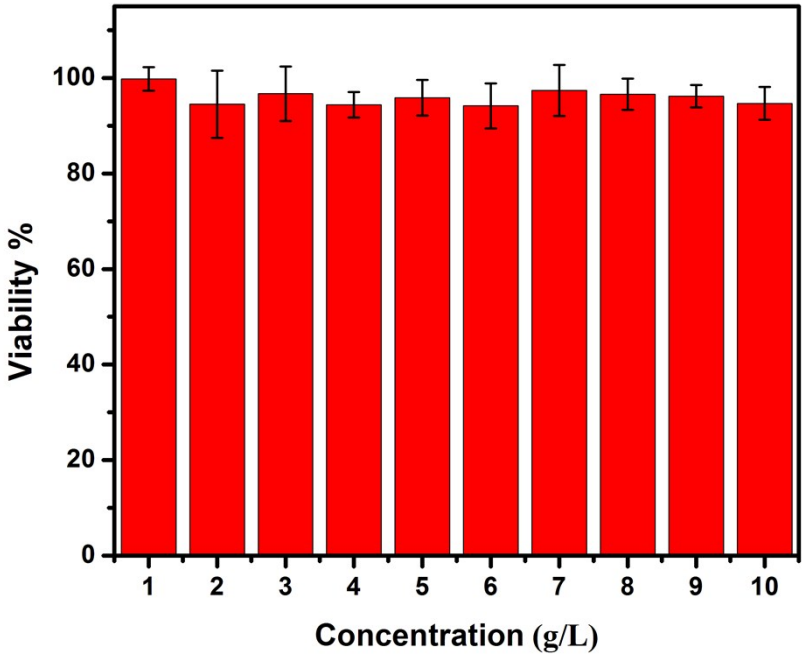

Figure S4. Cytotoxicity test of AuNCs on A549 cells.