Wavelength-tunable luminescent gold nanoparticles generated by cooperation ligand exchange and their potential application in cellular imaging

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Experimental Section

Reagents and Materials.

Chloroauric acid ($HAuCl_4$•$4H_2O$), sodium hydroxide (NaOH), trisodium tetraborate and glutathione (GSH) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Tetrakis(hydroxymethyl)phosphonium chloride (THPC) was purchased from TCI Development Co., Ltd (Shanghai, China). 11-mercaptoundecanoic acid (11-MUA), D-penicillamine (DPA), and thiazolyl blue tetrazolium bromide (MTT) were obtained from Sigma-Aldrich Company (USA). 3-mercaptopropionic acid (MPA) was obtained from Acros Organics (Shanghai, China). L-cysteine was purchased from Alfa Aesar Co., Ltd (Tianjing, China). Fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM) were purchased from Gibco (USA). All other reagents and solvents used were of analytical grade and used as received. Double distilled water was used throughout.

Synthesis of gold nanoparticles.

The gold nanoparticles were synthesized through reduction of $HAuCl_4 \cdot 4H_2O$ with THPC. In brief, NaOH (0.5 mL, 1 M) was added to aqueous solution (46 mL), followed by the addition of THPC solution (ca. 80% in water, 12 μL). The resulting THPC solution (5840 μL) was stirred 5 minutes, then followed by the rapid addition of an aqueous solution of tetrachloroauric acid ($HAuCl_4$•$4H_2O$, 160 μL, 9 mg/mL which was measured by ICP). Then the solution was stirred for 15 minutes for the further application.

Preparation of luminescent gold nanoparticles.

The gold nanoparticles were self-assembled with 11-MUA and DPA by introducing the stock solution (100 mM) into the as-prepared gold nanoparticles. In brief, to a 1.5 mL vial, the following reagents were added in order: water, trisodium tetraborate (100 μL, 50 mM, pH 9.2), as-prepared gold nanoparticles (500 μL), DPA stock solution (100 mM) and 11-MUA stock solution (100 mM) with different concentration ratios. The mixture solution was left to react for 3 days in the dark at room temperature. The resulting gold nanoparticles exhibited luminescence and can be observed directly upon a UV-lamp. The products were purified with centrifugation filtration using Nanosep filters (Pall Nanosep) with a molecular weight cut-off of 10 kDa (membrane nominal pore size ~ 1

nm) or high-speed centrifuged (16500 rpm). The sample was freeze dried and stored at 4 ℃ for future use.

Name	Ratio (11-MUA:DPA)	As-prepared Au NPs	Trisodium tetraborate	11-MUA (100 mM)	DPA (100 mM)	H_2O
Au-1	1:0	500 μL	100 μL	100μ L	0 μL	300 μL
Au-2	1:1	500 μL	100 μL	100 μL	100 μL	200 μL
Au-3	1:2	500 μL	100 μL	100 μL	200 μL	100 μL
Au-4	1:3	500 μL	100 μL	100 μL	300 μL	0 μL

Quantum yield

Luminescence quantum yield (QY) is defined as the numbers of emitted photos per number of absorbed photos. QYs are typically measured by a relative comparison method.

$$\frac{Q_t}{Q_s} = \frac{I_t A_s \eta_t^2}{I_s A_t \eta_s^2} = \frac{(\frac{I_t}{A_t})\eta_t^2}{(\frac{I_s}{A_s})\eta_s^2}$$

Q = Quantum yield, t = Test sample, S = Standard sample, η = Refractive index of solvent, A = Absorption at the selected excitation wavelength.

To calculate the QY absorption and luminescence spectra of concentration series of the test sample and a reference sample (with known QY) were measured. In this work, Rhodamine 6G dissolved in ethanol (Q = 95%) was used as a standard. Luminescent gold nanoparticles dispersed in water were used. For Au-1, Au-2, Au-3 and Au-4, concentration the absorption at 375 nm, 385 nm, 390 nm, 395 nm and the corresponding luminescence spectra were recorded, respectively.

Nanocluster characterization

Ultraviolet–visual adsorption spectra were measured by a UV–vis absorption spectrometer (UV759, China), and luminescence spectra were taken on a Cary Eclipse spectrofluorometer (Varian, USA). A luminescence lifetime spectra were obtained from a FLS-920 Spectrometer (Edinburgh Instrument, England). Transmission electron microscopy (TEM) images were taken on a JEOL JEM-2010 electron microscope (Japan) with an accelerating voltage of 200 kV. High resolution transmission electron microscopy (HRTEM) images were taken on a JEOL JEM-2100F electron microscope (Japan) with an accelerating voltage of 200 kV. The infrared spectra were obtained from a FTIR (Fourier Transform Infrared Spectrometer, Nicolet, Avatar-360, USA). Zeta potential experiments were carried out on a zeta potential meter (Malvern Zetasizer Nano, Malvern, UK). X-Ray photoelectron spectroscopy (XPS) experiments were carried out on a RBD upgraded PHI-5000C ESCA system (Perkin Elmer) with Mg Kα radiation (hν = 1253.6 eV) or Al Kα radiation (hν = 1486.6 eV). In general, the X-ray anode was run at 250 W and the high voltage

was kept at 14.0 kV with a detection angle at 54°. All spectra were referenced to the C1s peak at 284.6 eV.

Cell cultivation

Human cervical carcinoma (HeLa) cells were routinely cultured at 37 ℃ in flasks containing Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) in a humidified atmosphere and with 5% CO_2 in a Thermo culturist. Cells were plated in a tissue culture flask with 100% humidity.

Cytotoxicity assay.

Cytotoxicity was evaluated by performing MTT assays in the HeLa cells. Cells were seeded at 5×10^3 per cell into a 96-well cell culture plate in DEME with 10% FBS at 37 ℃ and with 5% CO_2 for 24 hours. Next, the cells were incubated with different concentrations (25 μg/mL - 200 μg/mL) of the nanoparticles for 24 hours. After that time, 100 μL fresh DMEM with 10% FBS and MTT (20 μL, 5 mg/mL in PBS) was added to each well, and the plate was incubated for another 4 hours at 37 ℃. MTT internalization was terminated by remove of the medium, then 150 μL DMSO was injected to each well. The assays were performed according to the manufacturer's instructions. The absorbance of MTT at 492 nm was measured by an automatic ELISA analyzer (SPR-960).

Cellular imaging

The HeLa cells were cultured in 12-well plates at a density of 10^5 cells/mL containing DMEM with 10% FBS at 37℃ and with 5% CO_2 for 24 hours, then the purified gold nanoparticles about 25 μg/mL were introduced into the culture dish containing the HeLa cells for 3 hours at 37 ℃ and with 5% CO_2. After washing with 1×PBS (10 mM phosphate buffered saline, NaCl 137 mM, KCl 2.7mM, NaH_2PO_4 10mM, KH_2PO_4 2 mM, pH = 7.4) three times, cells were fixed with 1 mL/well, 4 wt% paraformaldehyde in PBS for 15 minutes. Subsequently, the cells were washed with PBS to remove the excess paraformaldehyde, the sample was embedded in PBS solution. Luminescence imaging was performed with a Leica TCS SP5 microscope and using a Leica application suite, advanced fluorescence confocal scanning system. A 40× dry objective lens was used. Excitation of the luminescent gold nanoparticles was performed with a laser at 405 nm, and emission spectra were collected using a wavelength range of 510 – 550 nm (green), 550 - 600 nm (yellow), 570 – 620 nm (glow) and 590 - 640 nm (red) for the wavelength-tunable luminescent gold nanoparticles, respectively.

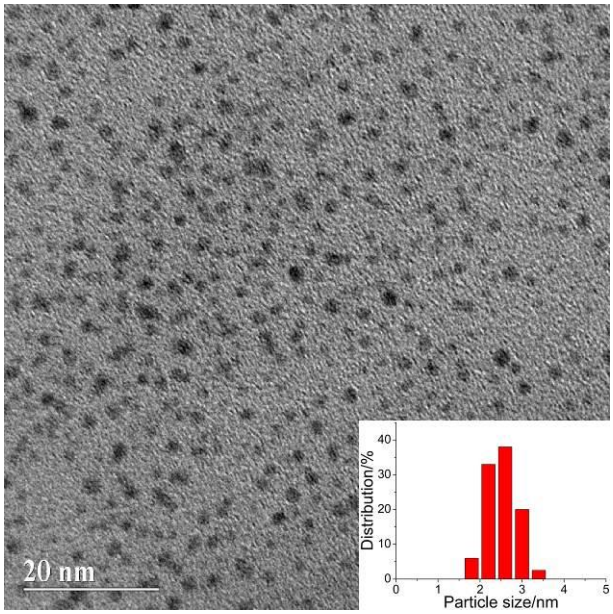

Fig. S1 TEM image and size distribution (insert image) of as-prepared gold nanoparticles.

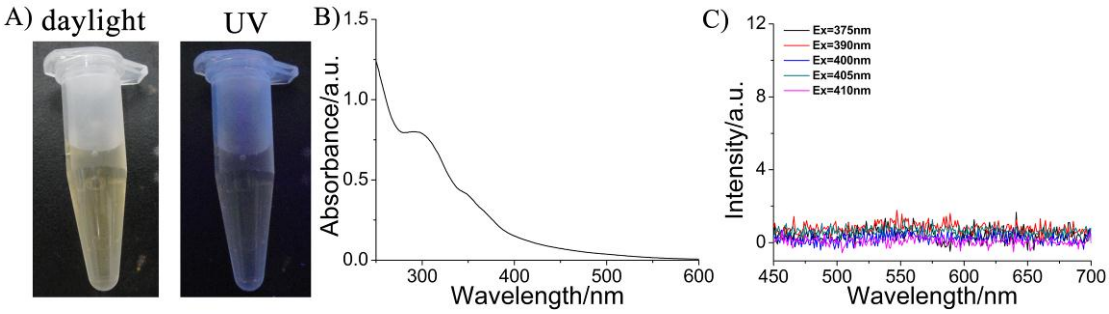

Fig. S2 A) Photographs of the as-prepared gold nanoparticles under daylight and UV-light. B) UV-vis absorption spectrum and C) luminescence spectra under different excitation wavelength of the as-prepared gold nanoparticles.

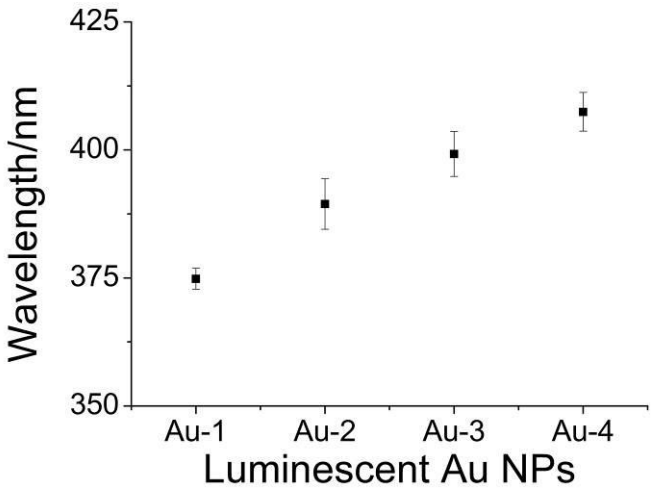

Fig. S3 UV-vis wavelength distributions of the wavelength-tunable luminescent gold nanoparticles.

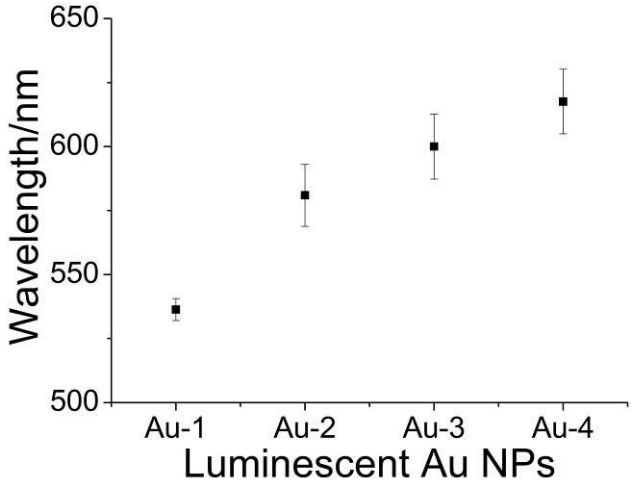

Fig. S4 Luminescence wavelength distributions of the wavelength-tunable luminescent gold nanoparticles.

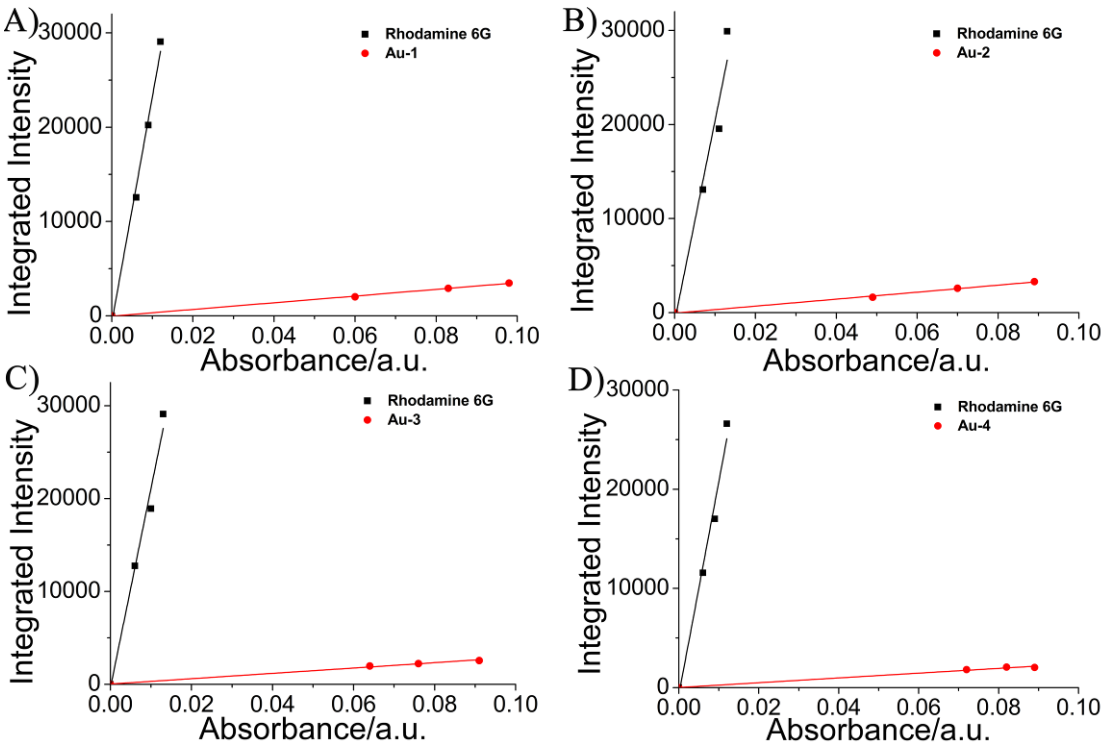

Fig. S5 Quantum yields of the wavelength-tunable luminescent gold nanoparticles. Excitation spectra are 375 nm, 385nm, 390 nm and 395 nm for A) Au-1, B) Au-2, C) Au-3, D) Au-4, respectively.

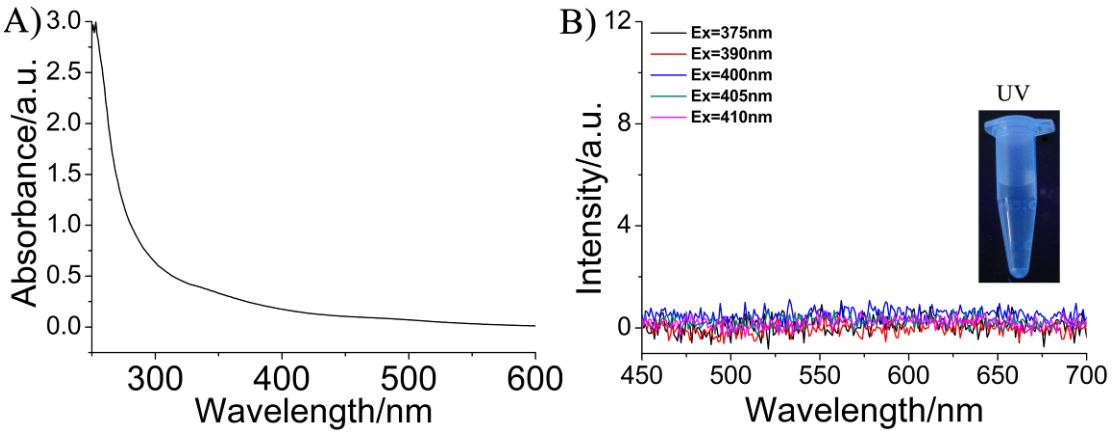

Fig. S6 A) UV-vis absorption spectrum and B) luminescence spectra under different excitation wavelength of the DPA coated gold nanoparticles. The insert shows the resulting products under UV-light.

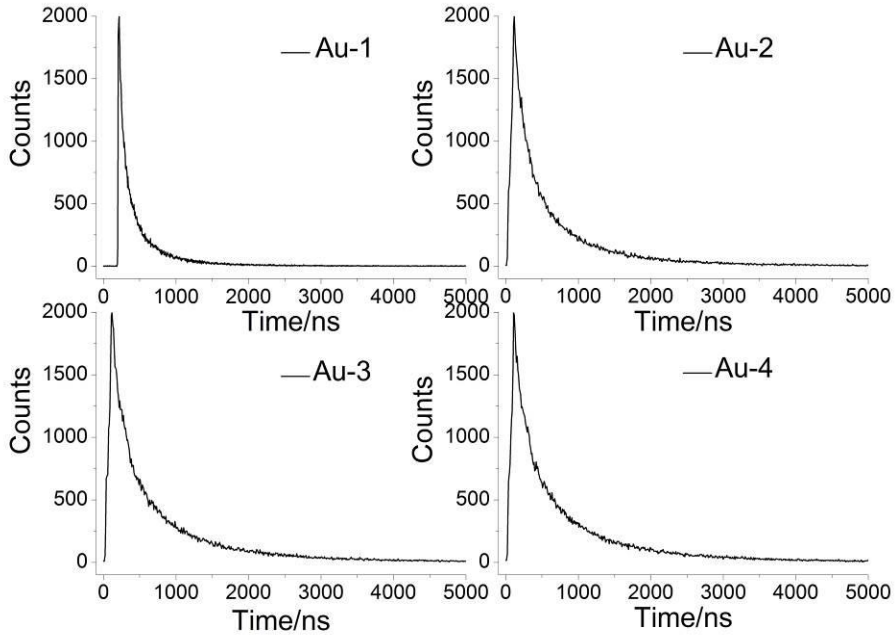

Fig. S7 Luminescence lifetimes, after excitation at 390nm, of the wavelength-tunable luminescent gold nanoparticles.

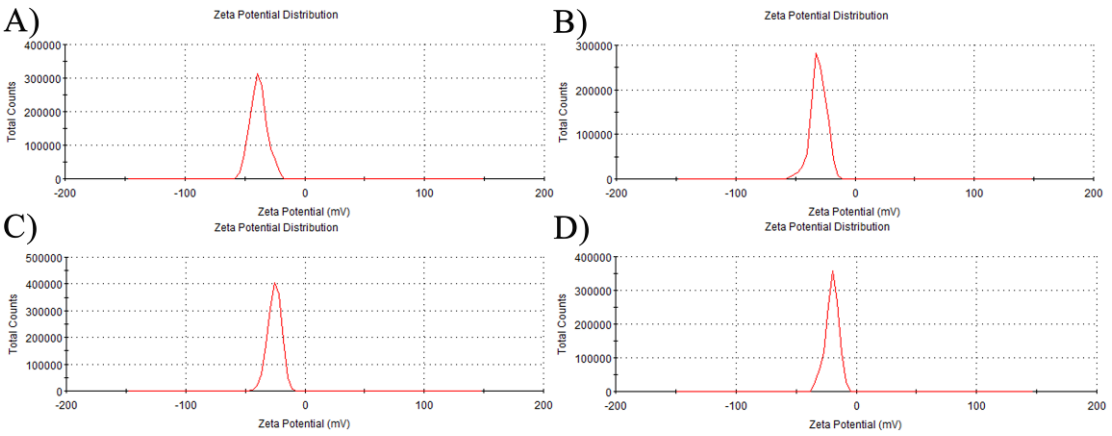

Fig. S8 Zeta potential of the wavelength-tunable luminescent gold nanoparticles in the double distilled water, A) Au-1, B) Au-2, C) Au-3 and D) Au-4.

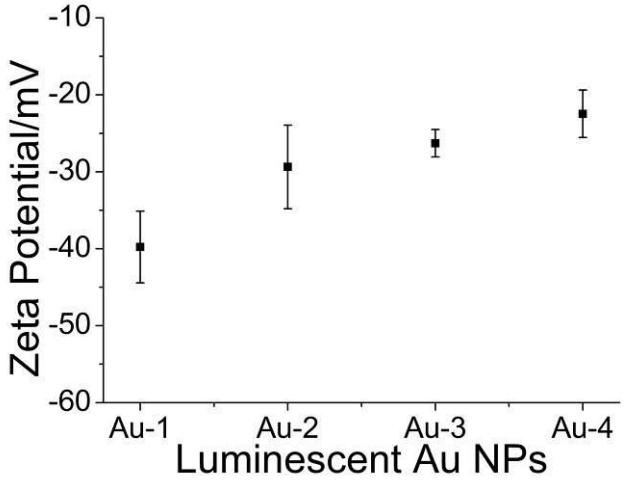

Fig. S9 Zeta potential distribution of the wavelength-tunable luminescent gold nanoparticles in the double distilled water.

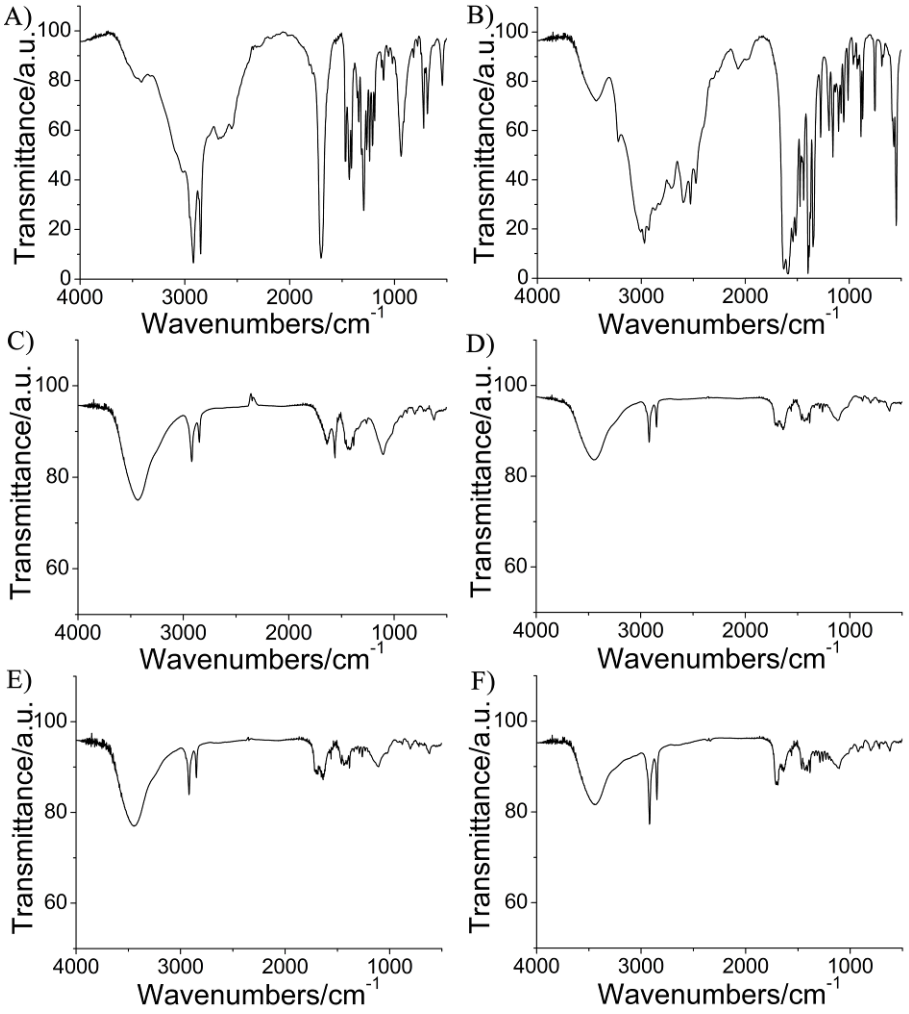

Fig. S10 FTIR spectra of the A) 11-MUA, B) DPA and wavelength-tunable luminescent gold nanoparticles, C) Au-1, D) Au-2, E) Au-3 and F) Au-4.

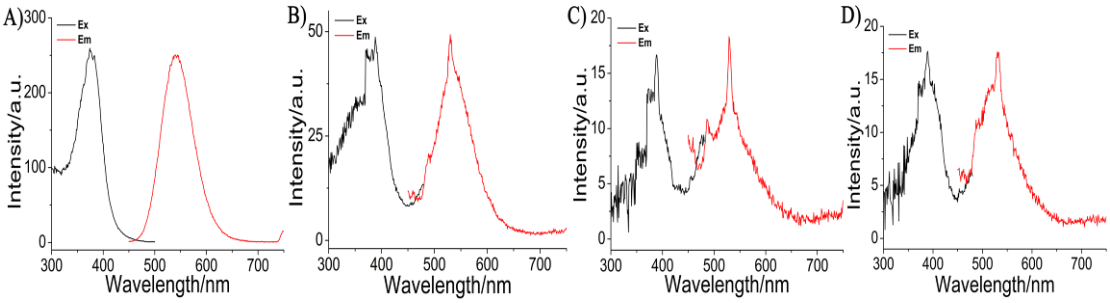

Fig. S11 luminescent spectra of the obtained gold nanoparticles**,** A), B), C), D) for the molar ratios of 1:0, 1:1, 1:2, 1:3 (11-MUA:GSH), respectively.

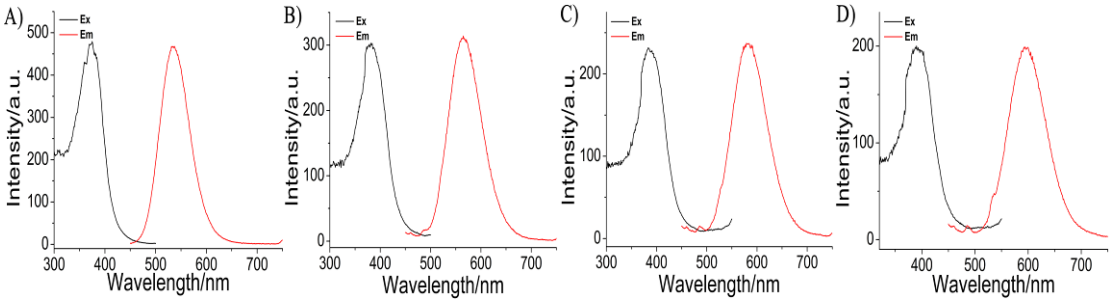

Fig. S12 luminescent spectra of the obtained gold nanoparticles**,** A), B), C), D) for the molar ratios of 1:0, 1:1, 1:2, 1:3 (11-MUA: L-Cys), respectively.

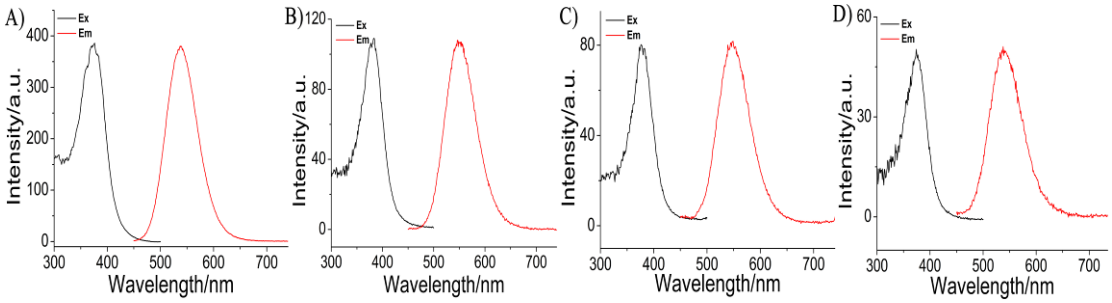

Fig. S13 luminescent spectra of the obtained gold nanoparticles**,** A), B), C), D) for the molar ratios of 1:0, 1:1, 1:2, 1:3 (11-MUA: MPA), respectively.

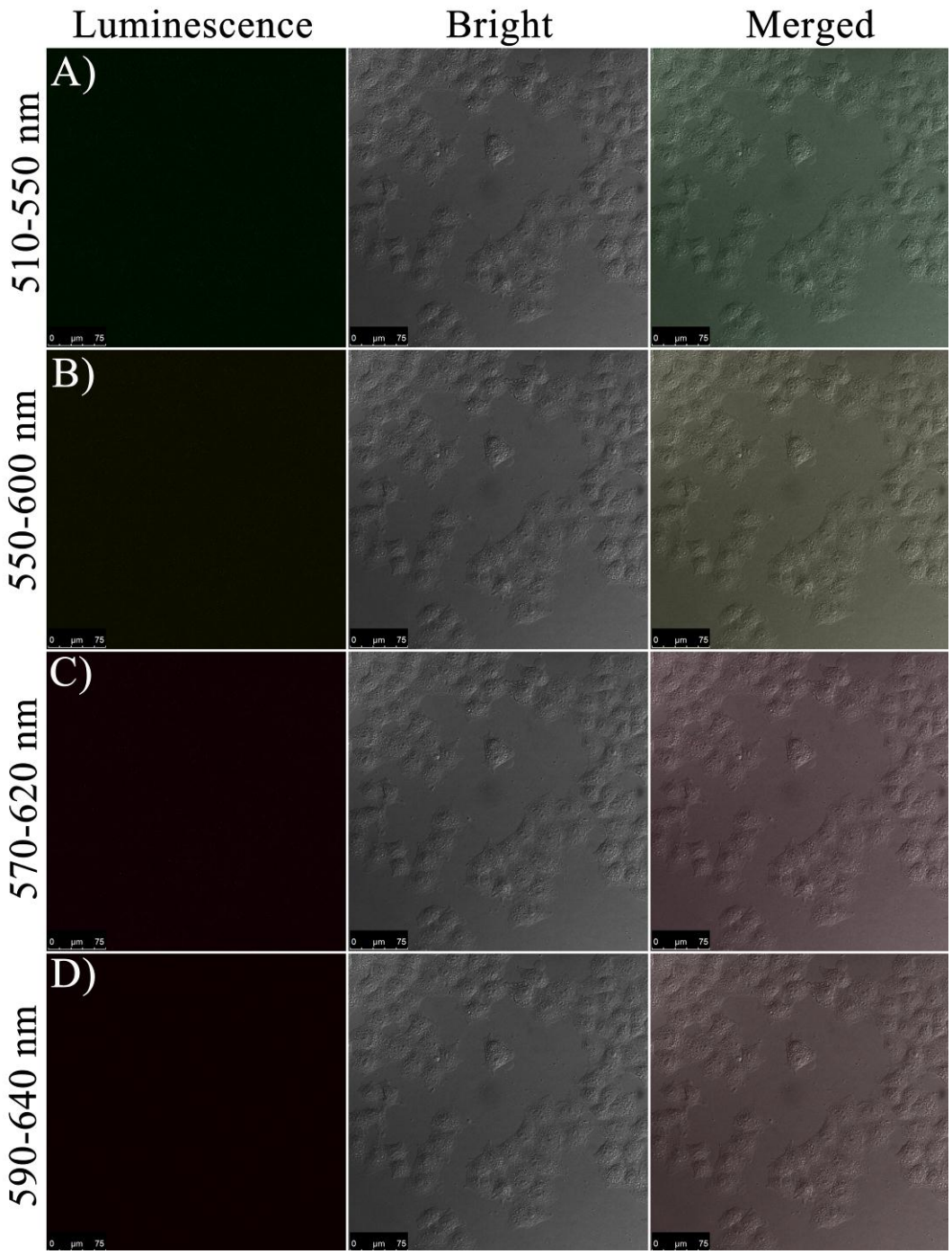

Fig. S14 Control experiment of the wavelength-tunable luminescent gold nanoparticles. A) 510 - 550 nm (Au-1), B) 550 - 600 nm (Au-2), C) 570 - 620 nm (Au-3) and D) 590 - 640 nm (Au-4).

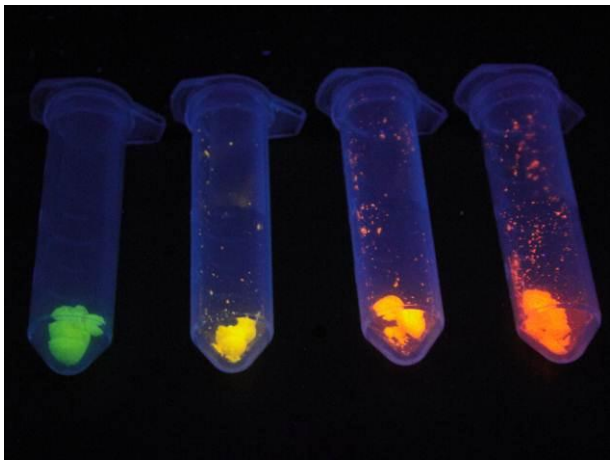

Fig. S15 Photographs of the freeze dried wavelength-tunable luminescent gold nanoparticles under a UV-lamp, which were used for XPS measure.