One-pot synthesis of biofunctional and near-infrared fluorescent gold nanodots and their application in Pb\textsuperscript{2+} sensing and tumor cell imaging

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Materials and methods

Reagents

All reagents were of analytical reagent grade. L-cysteine, hydrogen tetrachloroaurate(III) trihydrate (HAuCl₄•3H₂O), 3-(4,5-diMethylthialzol-2-yl)-2,5-diphe-nyl-etrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich (Milwaukee, USA). Sodium hydroxide (NaOH) was purchased from Beijing Chemicals (Beijing, China). For all aqueous solutions, high purity deionized water from a Millipore system (Millipore, Bedford, MA, USA) was used.

Instruments

An advanced microwave digestion system was used for the microwave pyrolysis (Milestone, USA). Fluorescence spectra was recorded on a PerkinElmer-LS55 fluorescence spectrometer. High-resolution transmission electron microscopy (HRTEM) images were taken on a JEOL TEM 2010 microscope operating at 200 kV. XPS measurements were performed using a Thermo ESCALAB VG Scientific 250 with monochromatized Al Kα excitation.

Synthesis of Cys-Au dots

All glass bottles were washed with aqua regia and rinsed with ultrapure water. In a typical MW-assisted synthesis experiment, 1.0 mL of 40 mg/mL Cys solution was added to 1.0 mL of 20 mM HAuCl₄ solution, followed by 100 μL of 1.0 M NaOH solution. Then, the mixture was under 250W microwave for 90 s. Thus, intensive orange fluorescent Cys-Au dots were achieved. The concentration of Cys-Au dots in our study was considered to be 10 mM by approximate.

Fluorescence Detection of Pb²⁺

3.0 mL of the as-prepared Cys-Au dots (10 mM,) was added to HAc-NaAc buffer (200 mM, pH = 4.5, 3.0 mL) containing various concentrations of Pb²⁺. The solution was mixed thoroughly and the
fluorescence quenching spectra was then recorded (excitation 355 nm; maximum emission 635 nm); the slit widths for the excitation and emission were set at 10.0 and 10.0 nm, respectively. The interfering effects of other metal ions were investigated individually in the presence of the fluorescent Au dots.

**Cell culture and cytotoxicity assay**

Hela and A549 cells obtained from the Institute of Cell Biology, Beijing Normal University (China) were used for cell experiments. Cells were cultured in Dulbecco’s modified eagle medium (DMEM, Invitrogen, Carlsbad, California), supplemented with 10% (v/v) fetal bovine serum (FBS) and maintained at 37 °C in a humidified atmosphere containing 5% CO₂. For the MTT toxicity test of Cys-Au dots, HeLa and A549 cells were seeded in a 24-well plate in cell medium overnight respectively. Subsequently, they were incubated with different concentrations of Cys-Au dots (0, 25, 50, 100, 250 and 500 μM) in cell medium for 2 h at 37 °C and 5% CO₂, respectively. Three replicate samples were prepared for each concentration. After removing Au dots-containing medium and washing twice with sterile PBS, cells were further incubated in cell medium for 24 h at 37 °C and 5% CO₂. Afterwards, cells were again washed twice with PBS, and 200 μL fresh medium plus 10μL MTT stock solution (12 mM in PBS) were added to each well. After incubation for 4 h at 37 °C and 5% CO₂, 400μL DMSO was added to each well and mixed thoroughly before finally measuring the absorbance of the solution at 360 nm.

**Imaging Cys-Au dots-stained HeLa and A549 cells**

HeLa and A549 cells were seeded in 8-well LabTek chambers (Nalge Nune International, New York) and allowed to adhere overnight at 37 °C and 5% CO₂, respectively. After removing the medium by twice washing with PBS, cells were incubated with 400μM Cys-Au dots in serum-free DMEM at 37 °C and 5% CO₂ for 2 h. Subsequently, cells were washed twice with PBS. Fluorescence imaging was
performed using a Two-Photo Laser Scanning Confocal Microscope-510 Meta with 63 × oil-immersion objective lens. Cys-Au dots were excited at 360 nm and observed through a band-pass filter (685 nm / 40 nm center wavelength/width, AHF, Tübingen, Germany).

**Fig S1** Fluorescence lifetime of the Cys-Au dots.
Fig S2 Infrared spectroscopy of the Cys-Au dots.

As shown in Fig S1, the infrared spectroscopy analysis of the Cys-Au dots further revealed that there were two main functional groups, –COOH and –NH₂, in the surface of the Cys-Au dots, with the fact: asymmetric and symmetric stretching vibration peaks of COO⁻ at 1600 and 1400 cm⁻¹ and the very broad band of NH₃⁺ stretch was observed in the 3000-3500 cm⁻¹ range. It is noteworthy that the band near 2550 cm⁻¹ which represented the -SH group, was not observed in the spectra. This demonstrated that cysteine combined to the surface of the Au dots by Au-S bonding, which was consistent with the previous report.

Fig S3. Influence of the synthesis conditions: (A) the reaction time, the concentration of (B) Cys, (C) HAuCl₄ and (D) NaOH on the fluorescent intensity of the Cys Au dots.
Fig S4. Photographs of the Cys Au dots obtained in the PBS buffer solutions of different pH under 365 nm UV light illumination.
**Fig S5.** Fluorescence spectra of the as-prepared Au dots (black line), after adding 20 μM Pb$^{2+}$ (red line), and followed by adding 100 μM EDTA (blue line). Insets are the corresponding photographs of (A) Cys-Au dots, (B) Cys-Au dots + 10 μM Pb$^{2+}$, (C) Cys-Au dots + 10 μM Pb$^{2+}$ + 100 μM EDTA under 365 nm UV light illumination.
Fig S6. Viability of HeLa cells after 24 h of incubation with different concentrations of Cys-Au dots in the cell medium as determined by an MTT assay. The error bars represent variations among three independent measurements.