Electronic Supplementary Information for

Au@Organosilica multifunctional nanoparticles for the multimodal imaging

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Experimental Section

Materials: 3-Mercaptopropyltriethoxysilane (MPS) was obtained from Alfa Aesar. Heterofunctional PEG (SH-PEG-COOH, MW=3000) was obtained from Rapp Polymers. 5,5'-Dithio-bis(2-nitrobenzoic acid) (Ellman's reagent), paraformaldehyde, fluorescent dye Hochest 33258, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich. Fluorescein 5(6)-isothiocyanate (FITC) was purchased from Fluka. Malachite green isothiocyanate (MGITC) and X-rhodamine-5-(and-6)-isothiocyanate (XRITC) were purchased from Invitrogen. Ammonia solution (NH_4OH, 25%), chloroauric acid ($HAuCl_4 \cdot 4H_2O$) were obtained from Sinopharm Chemical Reagent Co., Ltd. Hydroxylamine hydrochloride ($HONH_3Cl$), trisodium citrate were purchased from Shanghai Reagents Co. (Shanghai, China). L-cysteine was obtained from Xiamen Tagene Biotechnology Co., Ltd. (Xiamen, China). Dimethyl sulfoxide (DMSO) was obtained from Xiamen Lulong Biotech Co., Led. (Xiamen, China).

The following buffer solutions were used: PBS buffer (KH_2PO_4/K_2HPO_4, 150 mM NaCl, pH=7.6), Tris-HCl buffer (0.25 M, pH=8.0).

Characterization: TEM studies were performed on a F30 high-resolution transmission electron microscope operating at 300 kV (Tecnai, Netherlands). The samples were prepared by dropping the sols of Au@organosilica nanoparticles onto 300-mesh carbon-coated copper grids. TEM with energy-dispersive X-ray spectroscopy (EDX) studies were performed on a JEM-2100 electron microscope operating at 200 kV (JEOL, Japan). Scanning electron microscope (SEM) images were taken with a field emission microscope (Leo1530, Germany) operated at an accelerating voltage of 20 kV. A droplet of the aqueous colloidal suspension was cast on a flat single crystal silicon slide. After drying at room temperature, the sample was used for SEM imaging. A ~5 nm gold layer were sputtered on the sample surface before measurement to improve the conductivity of Au@organosilica colloids to obtain a good contrast. FT-IR spectra were collected on Avatar 330 FTIR Spectrometer (Thermo Nicolet, USA) using KBr pellets. UV-vis spectra were collected on UV-2100 Spectrophotometer (Shimadzu, Japan). Fluorescence emission spectra were recorded with a F7000 (Hitachi, Japan). Dark-field images, normal Raman spectra and SERS spectra from cells were obtained on a modified Renishaw inVia Raman Microscope with Leica DMI 3000M inverted microscope. A 785 nm diode laser was used for normal Raman measurement and the 632.8 nm laser line from a He-Ne laser was used for the SERS measurement. A long working distance 50× objective (NA=0.55, BD) was used for dark-field imaging and a 100× objective (NA=0.75, BD) was used for SERS measurement. The laser

spot was ~2 μm in diameter. An acquisition time of 10 s was used to obtain SERS spectra with a high signal-to-noise ratio. Multiplex detection in the selected cell region was characterized on an upright Renishaw inVia Raman Microscope equipped with a motorized x-y stage. A He-Ne laser (632.8 nm wavelength) and a 100× objective (NA=0.95) objective were used. The scanning step was 2 μm in the x and y directions, and the collection time was 10 s for each step. Confocal imaging of cells was performed with TCS SP5 laser scanning confocal microscope (CLSM) (Leica, Germany) and a 100× oil-immersion objective lens (NA=1.40-0.70) was used. Cells loaded with FITC-MGITC Au@organosilica nanoparticles were excited at 488 nm by using a multiline argon ion laser. Emission was collected from 500 to 600 nm. Hoechst 33258 was excited at 405 nm by using a multiline diode laser and the emission was collected from 415 to 485 nm.

Preparation of Au@organosilica nanoparticles:

Preparation of uniform 55 nm Au nanoparticles: The Au nanoparticles were synthesized using the previously reported seed-mediated growth method.[1] The 35 nm Au seeds were prepared by reduction of $HAuCl_4$ using sodium citrate in an aqueous solution according to the Frens' method. Further growth of the seeds to monodisperse 55 nm Au nanoparticles was carried out by adding $HONH_3Cl$ (25 mM, 30 mL) into the 50 mL 35 nm Au sol (~1.4×10^{11} NPs/mL) while stirring, followed by the addition of $HAuCl_4$ (2.5 mM, 30 mL) into the above solution under stirring at room temperature for several minutes. The final size expected from the feed solutions can be calculated according to the equation of previous work.[1]

Preparation of organosilica shell: SH-PEG-COOH (100 μM, 150 μL) solution was added into 4 mL sol containing 55 nm Au nanoparticles (~6.3×10^{10} NPs/mL), the mixture was shaken for 3 minutes. The solution was kept static for 10 minutes. Then different volume (0.5, 1, 2, 4, 6 and 8 μL) of MPS was added into the solution without dilution and the mixture was shaken for another 3 minutes. The solubility of MPS in the aqueous solution is very low and tends to form an emulsion. NH_4OH was then added to adjust the solution pH to 8.5-9. The mixture was then placed static (without any stirring or shaking) for 48 h to obtain Au@organosilica nanoparticles. The synthesis can be done without stirring or shaking is the unique advantage of simplicity of the present method. But we do not exclude the use of stirring or shaking. The sol was centrifuged and redispersed with ultrapure water for three times. The thickness of organosilica shell can be easily controlled by the amount of the MPS added.

Preparation of SERS probe of MGITC or XRITC labelled Au@organosilica nanoparticles: 4 μL

MGITC or XRITC (10^{-5} M) was added in to 4 mL 55 nm Au nanoparticles and the mixture was let static for 10 minutes. Organosilica shell was then coated on the MGITC or XRITC labelled Au nanoparticles following the standard procedure of "preparation of orgnaosilica shell" as mentioned above.

Preparation of FITC-MGITC (or XRITC) labelled Au@organosilica nanoparticles: 20 μL FITC (5 mg/mL, ethonal) was added into 1 mL MGITC (or XRITC)-Au@organosilica nanoparticles and incubated for 1-3 days. After incubation, the reaction mixture was centrifuged for more than 8 times to remove unbound reagents. The particles were then washed with 75% ethanol and then with asepsis water more than 10 times to remove ethanol.

Ellman's reaction: The free –SH groups of Au@organosilica nanoparticles were determined by Ellman's reaction, according to the literature.[2] Briefly, the obtained Au@organosilica nanoparticles were washed by centrifugation for three times and redispersed with Tris-HCl buffer (0.25 M, pH 8.0). 200 μL Au@organosilica suspension in Tris-HCl buffer was then added to 1000 μL of Ellman's reagent (0.1 mM) to react for 10 minute at room temperature. Then the absorbance of the mixture was measured at a wavelength of 412 nm by a UV–visible spectrophotometer. The amount of thiol moiety was calculated from the corresponding standard curve elaborated between 0 and 0.5 mM of L-cysteine (r^2 =0.9995). The free –SH species in the Au@organosilica nanoparticles was determined to be about 45 μM.

Cell culture: HeLa cells (ATTC CCL2) were cultured in DMEM (high glucose) (Hyclone) supplemented with 10% serum (Hyclone) and 1% penicillin/streptomycin (Xiamen Lulong Biotech Co., Ltd. Xiamen, China). The cells were maintained at 37 °C in a humidified 5% CO_2-containing atmosphere.

MTT assay: The biocompatibility and cytotoxicity behavior of FITC-MGITC Au@organosilica nanoparticles was studied on HeLa cells by using the MTT assay. For this purpose, HeLa cells were seeded at a density of 1×10^5 cells per well in polystyrene 96-well culture plates, and incubated for 24 h till the 70% confluence. After the culture medium was removed, 100 μL of serum free DMEM medium containing Au@organosilica nanoparticles with different shell thickness (25-300 μg/mL) were added into each well. Cells treated with medium only served as a negative control group. After 24 h co-incubation, 20 μL of MTT solution (5 mg/mL in PBS buffer) was added into the medium and further cultured for 4 h. At the end of assay, MTT solution was removed and 100 μL of DMSO was added to dissolve the blue formazan crystal produced by proliferating cells. The plate was incubated

for an additional 10 min before determination at 490 nm in a spectrophotometric microplate reader (Bio-tek ELX800, USA). All experiments were performed in quadruplicate, and the relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

Cellular uptake: HeLa cells (1×10^5) were first seeded onto sterile cover glasses in a 6-well plate and cultured overnight to about 60% confluence in a complete DMEM, then the cells were treated with FITC-MGITC labelled Au@organosilica nanoparticles (30 μg/mL) in a serum-free DMEM for 12 h. Afterwards, the sample was washed twice with PBS for 10 min and fixed in paraformaldehyde (4% in PBS) for 20 min at room temperature. After washed three times with PBS, the solution was added with Hochest 33258 (10 μg/mL) and maintained for 20 min for nucleus staining and then washed three times with PBS.

S1. SEM characterization of Au@organosilica nanoparticles of different shell thicknesses

The SEM images show that Au@organosilica nanoparticles with different shell thicknesses are monodispersed in size from a large scale. SEM images shown as insets obtained at a higher magnification reveals that the surface morphology change from rough to smooth with the increasing shell thickness, which is similar to the results of $Au@SiO_2$.[3]

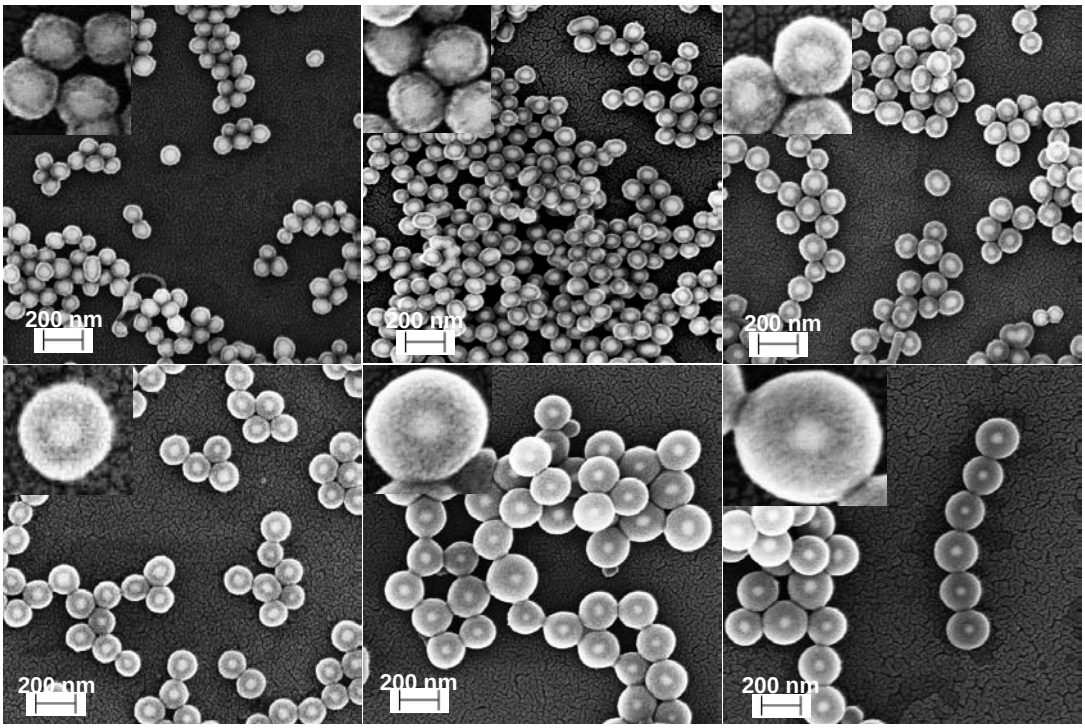

Fig. S1**.** SEM images of Au@organosilica nanoparticles with the 55 nm Au core and different shell thicknesses: (a) 15nm, (b) 20 nm, (c) 30 nm, (d) 45 nm, (e) 60 nm and (f) 75 nm. The insets show the nanoparticles at a high magnification.

S2. In situ monitoring the S-S bond formation of Au@organosilica nanoparticles.

To reveal the mechanism accompanying the formation of the organosilica nanoparticles, a roughened Au electrode was used to simulate the Au nanoparticles system by providing stronger signals. In brief, a Au electrode was firstly roughened by oxidization reduction cycles (ORC) using the Weaver's method[4]. Then, it was immersed into a 8 mM/L MPS solution for 2.5 h for adsorption. We could not observe the band at 509 cm^{-1} assigned to S-S vibration before the hydrolysis process. However, the peak becomes apparent at about 2.5 h after addition of NH_4OH to the MPS solution initiate the hydrolysis process, which clearly demonstrates the formation of S-S bond. This band increases synchronically with other bands of the MPS chain.

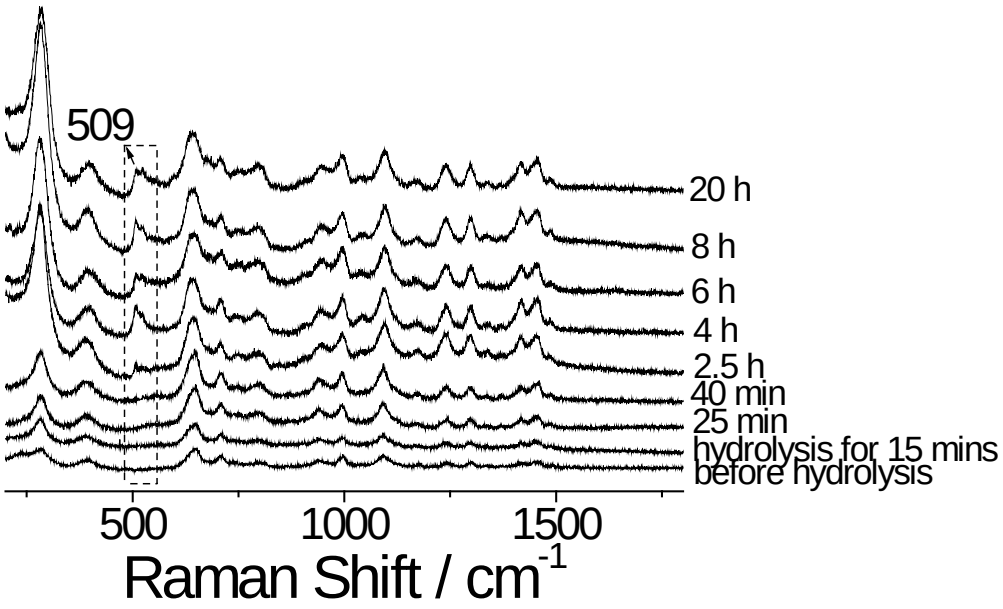

Fig. S2. SERS spectra of MPS without hydrolyzation monitor the hydrolysis process of MPS and the formation of S-S stretching band by using roughened Au electrode.

S3. SEM and TEM characterization of MGITC labelled Au@oragnosilica nanoparticles.

Figure S3 shows the SEM and TEM images of MGITC labelled Au@organosilica nanoparticles with different thicknesses of the organosilica shell. The dose of MPS was the same as that for synthesizing the pure Au@organosilica nanoparticles. At a thin shell thickness (up to 30 nm), there is only one Au nanoparticles. However, we can find multiple Au nanoparticles in one MGITC labelled Au@organosilica nanoparticle, indicating aggregation of the Au core occurs. The aggregation is highly probably induced by the high concentration of MPS. Therefore, in the present work, we prefer using the thickness of 30 nm for imaging purpose. A stepwise addition of MPS can successfully overcome

the aggregation problem for preparing MGITC labelled Au@organosilica nanoparticles with thicker shells

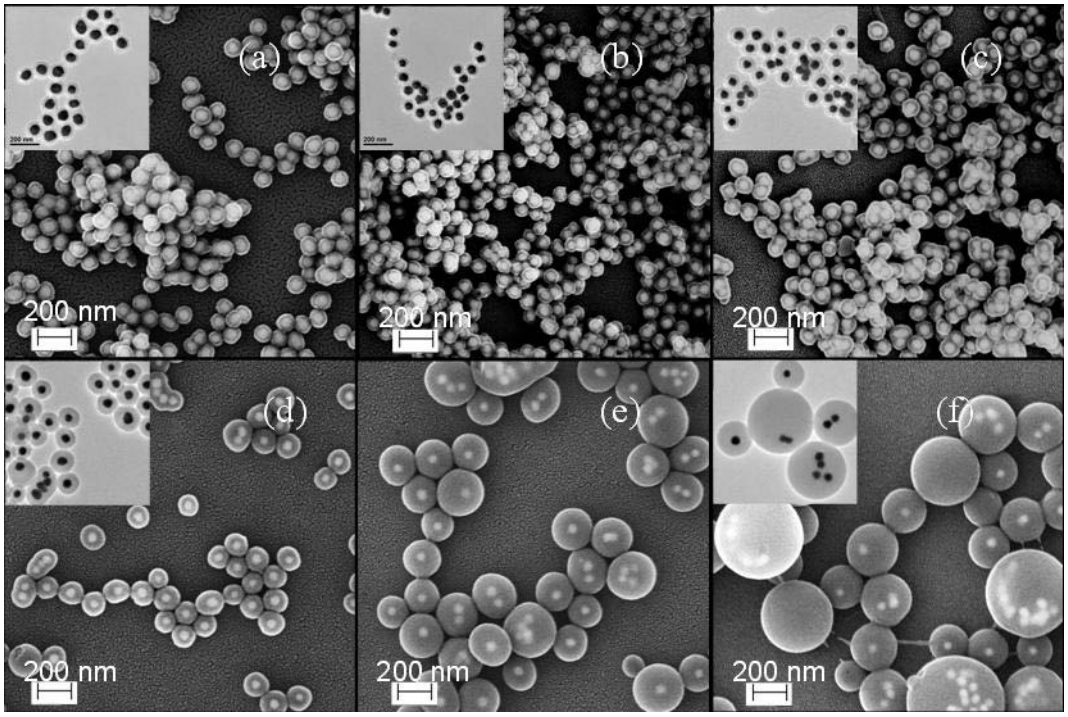

Fig.S3. SEM and TEM images of MGITC labelled Au@organosilica nanoparticles with the 55 nm Au core and different shell thicknesses: (a) 15nm, (b) 20 nm, (c) 30 nm, (d) 45 nm, (e) 60 nm and (f) 75 nm. The insets show the TEM images of the labelled nanoparticles.

S4. Dye releasing profile of FITC labelled Au@oragnosilica nanoparticles.

FITC labelled Au@organosilica nanoparticles with a shell thickness of 45 nm were used for the the dye releasing test. Briefly, the fluorescent signal of FITC labeled Au@organosilica nanoparticles right after preparation was measured firstly. After leaving the nanoparticles in water for a given time, 1 mL of the colloid was taken out and centrifuged. The obtained precipitate was re-dispersed in 1 mL of water and the fluorescent signal was measured again. As shown in Figure S4, the fluorescent intensity of nanoparticles decreased by approximately 10% for 8 h and 13% for 24 h. The slight decrease of the intensity may be attributed to the loss of nanoparticles during the centrifugation as well as the release of dye. The result confirms that the CLSM images were mainly contributed by FITC molecules trapped in the orgnosilica shell rather than the free FITC molecules. The covalent bond between free thiol group and isothiocyanate group of FITC may account for the good stability.

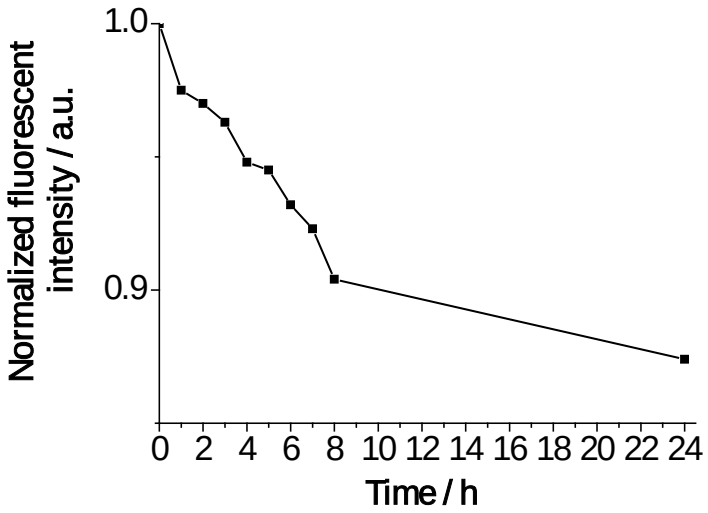

Fig. S4. Fluorescence releasing curve of FITC labeled Au@organosilica nanoparticles normalized with the original intensity of the nanoparticles.

1. P. P. Fang, J. F. Li, Z. L. Yang, L. M. Li, B. Ren and Z. Q. Tian, *J. Raman Spectrosc.*, 2008, **39**, 1679-1687.
2. G. L. Ellman, *Arch. Biochem. Biophys.*, 1959, **82**, 70-77.
3. J. Ye, B. V. de Broek, R. De Palma, W. Libaers, K. Clays, W. Van Roy, G. Borghs and G. Maes, *Colloid Surf. A-Physicochem. Eng. Asp.*, 2008, **322**, 225-233.
4. P. Gao, D. Gosztola, L. W. H. Leung and M. J. Weaver, *J. Electroanal. Chem. Interfacial Electrochem.*, 1987, **233**, 211-222.