Supporting Information for

Water-dispersible near-infrared Ag_2S nanoclusters with tunable fluorescence for bioimaging application

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1. Experimental Section

Chemicals and Materials: D-penicillamine (DPA) was obtained from Alfa Aesar (Tianjin, China). $AgNO_3$ and other reagents were purchased from Beijing Chemical Works (Beijing, China). Water used throughout all experiments was purified with the Millipore system (18.2 MΩ). All glassware was washed with *aqua regia* and rinsed with ultrapure water.

Apparatus and characterization: High-resolution transmission electron microscope (HRTEM) was recorded on JEM-2100F operated at 200 kV. X-ray photoelectron spectroscopy (XPS) was investigated by using ESCALAB-MKII 250 photoelectron spectrometer (VG Co.) with Al Kα X-ray radiation (1486.6 eV) for excitation. IR spectra were taken on a VERTEX Fourier transform infrared spectrometer (Bruker). X-ray power diffraction (XRD) pattern was recorded on a D8 Focus diffractometer (Bruker) with a Cu Kα radiation source (λ= 0.15406 nm). Fluorescence spectroscopy was collected with a Fluoromax-4 spectrofluorometer (Horiba Jobin Yvon Inc. France) using 5 nm/ 5 nm slit widths of excitation and emission. UV-vis absorption spectra were obtained using a Cary 50 Scan UV-visible spectrophotometer (Varian, USA). The luminescence decay curves were detected by a Lecroy Wave Runner 6100 Digital Osilloscope (1 GHz) using a tunable laser (pulse width = 4 ns, gate = 50 ns) as the excitation (Contimuum Sunlite OPO).

Synthesis of Ag_2S NCs: In a typical procedure, $AgNO_3$ aqueous solution (1.6 mL, 0.1 M) was added to 38.4 mL DPA (0.0191 g) solution in 250 mL beaker. After stirring at room temperature for 20 min, the mixture was heated in a domestic microwave oven (800W, Galanz) for 3 min. Finally, the product was collected and additional water was added to make the final volume 40 mL. The as-prepared Ag_2S NC (denoted as A2) was used directly without further treatment. To prepare Ag_2S NCs with longer emission wavelength, additional amounts of 0.1 M $AgNO_3$ aqueous solution (40 μL for A3, 60 μL for A4) was added to 4 mL the as-prepared A2, respectively. And then the mixture was kept stirring for 8 h at room temperature. For A1, $AgNO_3$ aqueous solution (0.4 mL, 0.1 M) was added to 9.6 mL DPA (0.006 g)

solution in 250 mL beaker. The mixture was directly subjected to microwave irradiation for 3 min.

MTT Assays and Cell Imaging: For the cell cytotoxicity test, HeLa cells (1×10^4 cells/well) were seeded on a Costar 96-well tissue-culture cluster and cultured at 37 ℃ with 5% CO_2 in an incubater overnight to adhere cells onto the surface. Then the cells were exposed to 100 μL of fresh Dulbecco's modified Eagles' medium (10% fetal bovine serum) containing Ag_2S NCs for another 24 h. A control experiment was performed in the absence of NCs. After adding 10 μL of MTT reagent (5 mg mL^{-1}) into each well, the cells were incubated for another 4 h. The precipitated formazan violet crystals were dissolved by 100 μL of dimethylsulfoxide (DMSO, Sigma). The cluster was vibrated for 10 min to completely liberate the crystals. Finally, the absorption at 490 nm was monitored using an EL808 ultra microplate reader (Bio TEK Instrument Inc.).

For the cell imaging experiment, HeLa cells (approximately 5×10^4 cells/well) were seeded in a 24-well plate and cultured at 37 ℃. The Ag_2S NCs aqueous solution (50 μL, 2 mg/mL) was mixed with the culture medium (500 μL) and then added to the wells. After incubation for 24 h, the HeLa cells were harvested using 0.25% trypsin/0.53 mM EDTA, washed three times with PBS (1 mL each time for three times), and kept in PBS for the optical imaging by a confocal microscope (Leica TCS SP2) with a 100 × objective.

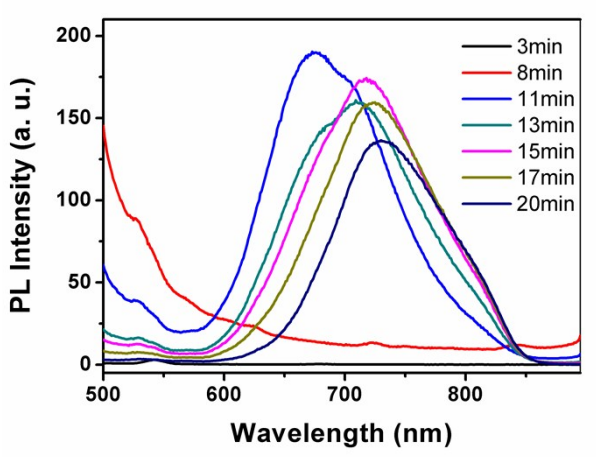

Fig. S1 The evolved fluorescent emission spectra of Ag_2S NCs prepared using hot plate as heating resource. The emission spectra showed significant scattering due to cloudy white Ag(I)SR thiolates aggregates.

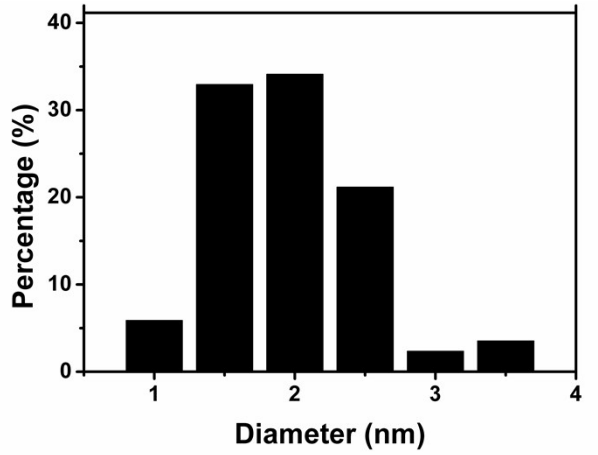

Fig. S2 The size distributions of the as-prepared Ag_2S NCs A2.

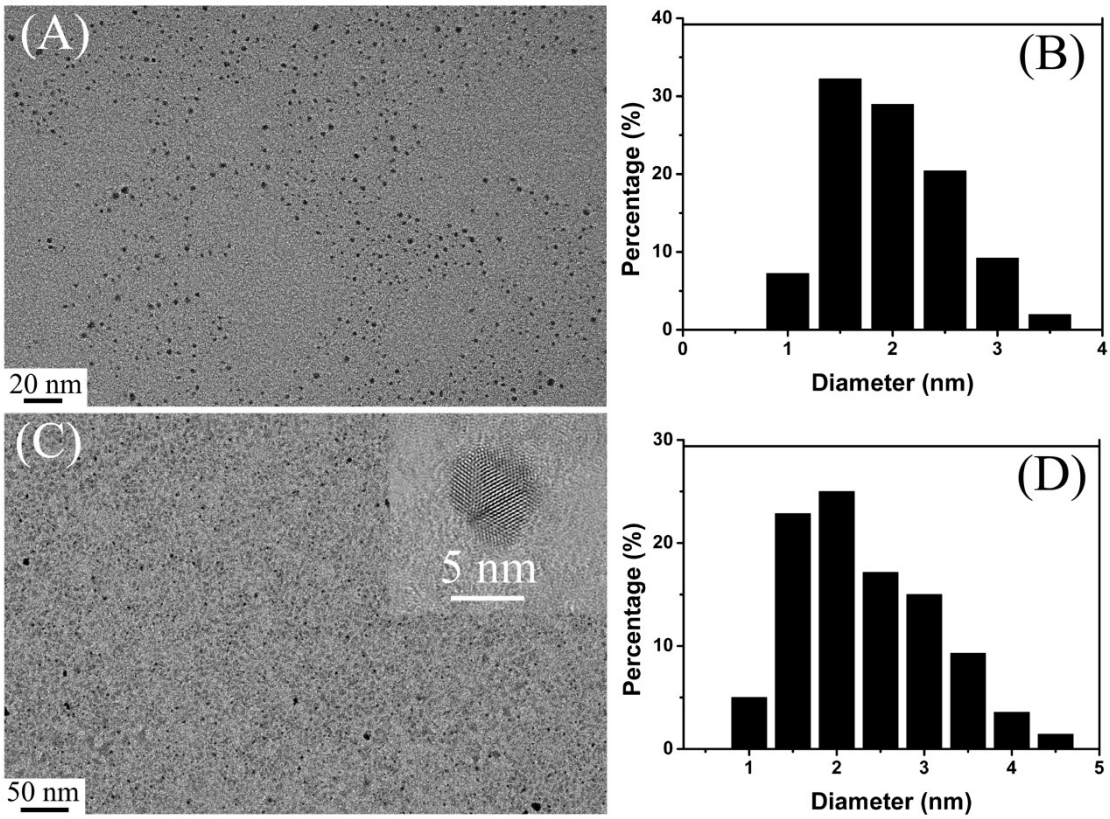

Fig. S3 The representative TEM images of the as-prepared Ag_2S NCs (A) A3 and (C) A4. Inset: HRTEM image of Ag_2S NCs A4. The size distributions of the as-prepared Ag_2S NCs (B) A3 and (D) A4.

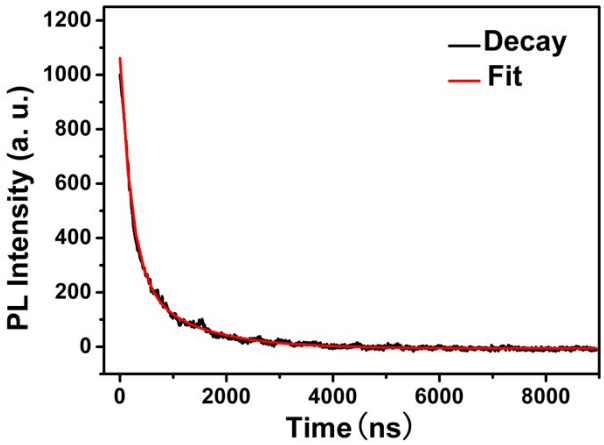

Fig. S4 PL decays (355 nm laser excitation, and monitored through 670 nm bandpass filter) of the Ag_2S NCs.

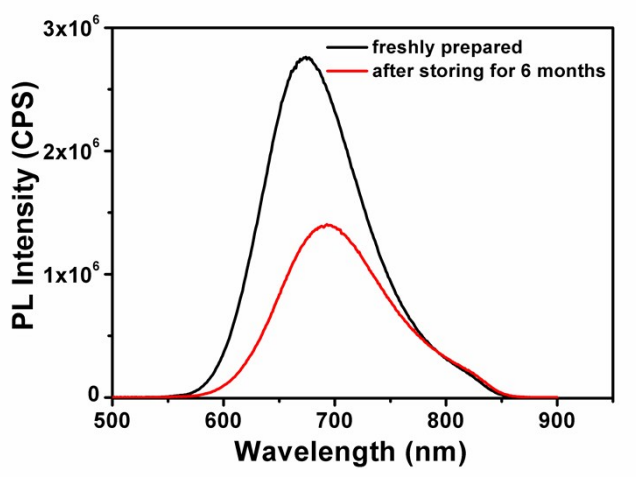

Fig. S5 The fluorescent emission spectra of the freshly prepared Ag_2S NCs and the Ag_2S NCs stroring for 6 months at ambient conditions under room light.

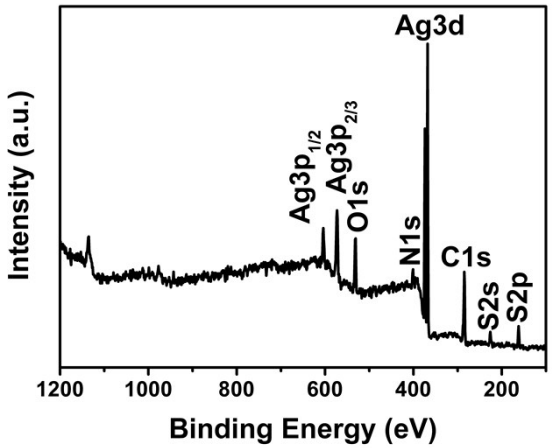

Fig. S6 XPS survey spectrum of the Ag_2S NCs.

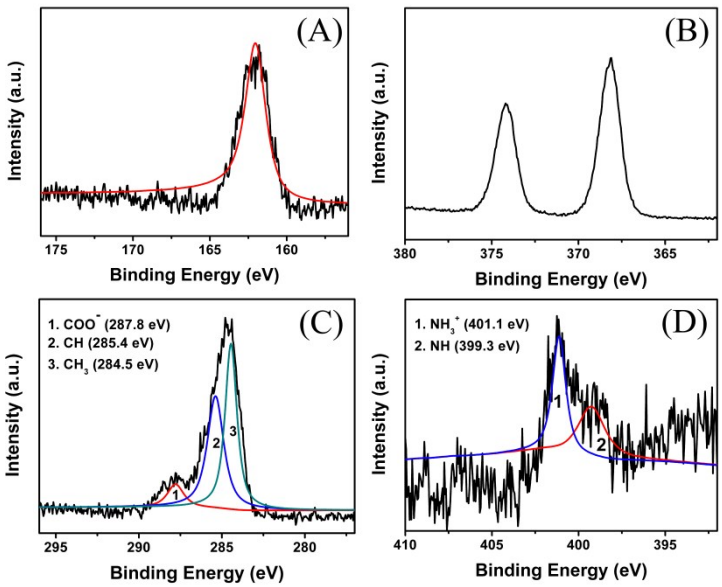

Fig. S7 XPS of the resultant Ag_2S NCs: (A) S 2p; (B) Ag 3d; (C) C 1s; (D) N 1s.

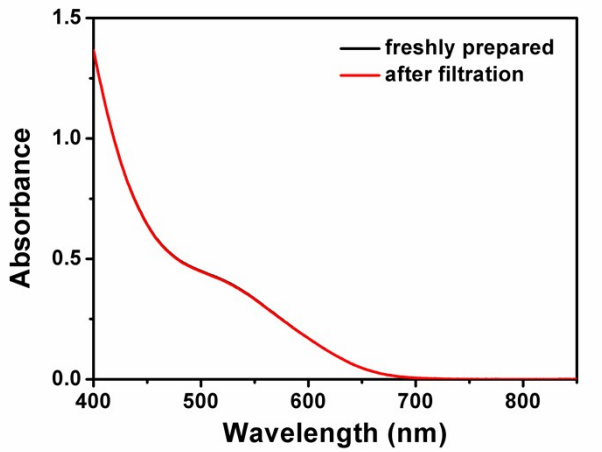

Fig. S8 The absorption spectra of the freshly prepared Ag_2S NCs and the filtered Ag_2S NCs after storing for 1 week at ambient condition.

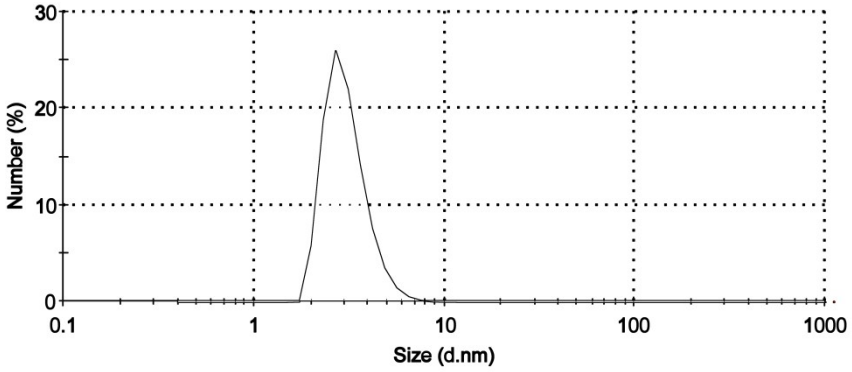

Fig. S9 The hydrodynamic size distribution of Ag_2S NCs in PBS buffer obtained from DLS measurements.

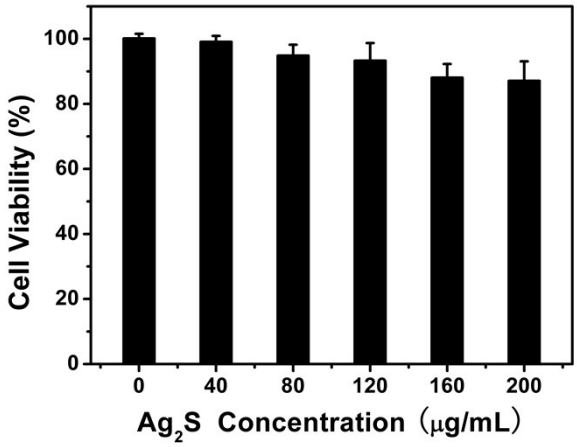

Fig. S10 Cytotoxicity of the resultant Ag_2S NCs toward HeLa cells.