Supporting Information

Ultrasonication-Promoted Synthesis of Luminescent Sulfur Nanodots for Cellular Imaging Applications

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1. EXPERIMENTAL SECTION

1.1. Materials. Sublimed sulfur (99.95%) and sodium sulfide nonahydrate (Na$_2$S·9H$_2$O) were purchased from Aladdin. Polyethylene glycol 400 (PEG-400) was obtained from Tianjin GuangFu Fine Chemical Research Institute (Tianjin, China).

1.2 Synthesis of S-dots. Sublimated sulfur (0.1g), water (40 mL), PEG-400 (2.5mL), and 7.0 g Na$_2$S·9H$_2$O were mixed in a 100 mL beaker. The mixture was allowed to react for a period of time (3 h~12 h) under ultrasonication. Intense green emission appeared under the radiation of UV-light, suggesting the formation of S dots.

1.3 CCK-8 assay. EAS-2B cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco (Carlsbad, CA, USA)) supplemented with 10% fetal bovine serum (Gibco) and 1% antibiotic-antimycotic (Gibco) in a humidified incubator at 37 ℃ with 5% (v/v) CO$_2$ and 95% (v/v) air. Cells (5×10^3/per well) were seeded in 96-well plate and grown for 24 h to reach 80-90% confluency. Cells were treated with fresh medium containing different volume of SQDs (1 μl, 5 μl, 10 μl, 15 μl, 20 μl, 25 μl and 30 μl) for 4, 8 and 24 h respectively. Washing the cells twice with phosphate-buffered saline solution (PBS). 100 μl CCK-8 solution (Meilunbio, Dalian, China) was then added and incubated for 2 h. The absorbance of each well was measured by a microplate reader. The number of viable cells in different groups was compared with that in the control group.

1.4 Confocal microscopy assay. EAS-2B cells (5×10^4/per dish) were seeded in a Live Cell Imaging Culture Dish (Meilunbio, Dalian, China) for 24 h. The cells were treated with fresh medium containing different concentrations of SQDs (1 μl, 5 μl, 10 μl, 15 μl, 20 μl, 25 μl and 30 μl) for 1 h. Washing the cells twice with PBS to remove the unbound SQDs. Bright field images and fluorescent images of cells were taken by a confocal microscopy (LSM800, Carl Zeiss, Germany) with excitation wavelength at 488 nm and emission wavelength at 509 nm.

1.5 Characterization. Spectrometer of UV-3600 (Shimadzu Japan) and F-7000 (Hitachi, Japan) were used to record the PL and UV-absorption spectra of S-dots. A FLS980 spectrometer (Edinburgh Instruments) equipped with an integrating sphere was employed to measure the time-resolved PL decay curves and absolute PL QY of S-dots. TEM images were acquired on a transmission electron microscopy (TEM; FEI Tecnai G2 F20 S-TWIN, FEI, USA). XPS spectra were collected on a photoelectron spectrometer (ESCALAB-MKII 250, Thermo, USA). Fourier transformed infrared (FTIR) spectra were recorded by a Nicolet IS10 FTIR spectrometer (Thermo, USA). The cell viability was calculated by comparing the absorption (450 nm) of 96-well plate by a microplate reader (Multiskan Go 1510, Thermo, USA) Ultrasonication treatment was carried out on an ultrasonic homogenizer (SCIENTZ-IID, SCIENTZ) working at 300 W.
Figure S1. UV-vis absorption spectra of S-dots synthesized by different ultrasonication time, under different times of dilution.

Figure S2. EDS spectrum of S-dots.

Figure S3. PLE spectra of S-dots synthesized by 3 h and 12 h ultrasonication.
Figure S4. PL spectra of S-dots synthesized by adding different volume of PEG, as indicated on the frame.

Figure S5. Full XPS spectrum of S-dots.
Figure S6. TEM image of S-dots synthesized without adding PEG.

Figure S7. PL spectra of S-dots synthesized by using different chain length PEG, as indicated on the top of frame.
Figure S8. PL spectra (excited at 420 nm) of S-dots synthesized by ultrasonication, direct heating using Na$_2$S and bulk sulfur, together with using bulk sulfur and NaOH as raw materials.