Supplementary Information

Aqueous synthesis of multidentate polymer-capping Ag_2Se quantum dots with bright photoluminescence tunable in second near-infrared biological window

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Experimental section

Materials

Silver nitrate ($AgNO_3$, 99.9 %), sodium borohydride ($NaBH_4$, 96 %), sodium selenite (Na_2SeO_3, 98 %), hydrazine hydrate ($N_2H_4 \cdot H_2O$, 80 %), indocyanine green (ICG, 98 %), dimethyl sulfoxide (DMSO, 97 %), sodium hydroxide (NaOH, 97 %), 2-Mercaptoethylamine (MEA, ~ 95 %), N-hydroxysuccinimide (NHS, 99 %), N,N'-diisopropylcarbodiimide (DIC, 98+ %), and *β*-mercaptoethanol (BME, 99+ %) were all purchased from Sigma Aldrich. Poly(acrylic acid) (PAA, $M_W \approx 2000$) was purchased from Aladin. Dulbecco's modified Eagle medium (DMEM) and Hanks balanced salt solution (HBSS) were provided by Beyotime. Phosphate buffered saline (PBS) solution (0.2 M, pH = 7.4) was prepared in our own lab. Hela cells and L929 cells (mouse fibroblast cells) were provided by Institute of Biochemistry and Cell Biology, CAS. The water used was deionized water.

Preparation of PAA-*g*-MEA ligand

In a typical procedure, 2 g of PAA (≈ 28 mmol based on carboxylic acids) was dissolved in 50 mL of DMSO, stirred at 40 °C for 24 h. MEA (0.74 g, 9.6 mmol) was dissolved in 20 mL of DMSO, which was mixed with the above solution. Then NHS (2 mg, 18 mmol) dissolved in 10 mL of DMSO was added, and the resultant mixture was protected from light and bubbled with nitrogen flow for 30 min at 40 °C. Subsequently, DIC (1.48 g, 11.6 mmol) was added dropwise over a course of 40 min under vigorous stirring and nitrogen flow. The reaction was allowed to proceed for 4 days at 50 °C in the dark. Afterward, 30 mL of piperidine was added, and the reaction solution was further stirred for 3 h to deprotect the primary amines. BME (1 g, 12.9 mmol) was added to quench the reaction, and the solution was stirred for another 2 h at 50 °C. Then the system was cooled to room temperature, centrifuged and filtered, followed by addition of 40 wt % NaOH solution to precipitate the polymer with ca. 23 % of the carboxylic acid functional groups modified with MEA portion. The precipitate was washed three times with hot DMSO (50 °C) and then with acetone at room temperature. After filtration, the product was dried in vacuum at room temperature (with yield of 83.5 % based on MEA), and stored in a vacuum dessicator.

Characterization

Fourier transform infrared spectroscopy (FTIR) tests were conducted on a Spectrum 100 FTIR spectrometer (PerkinElmer, US). Samples were dried, powdered, and made into films by mixing them with KBr. Sample micrographs were recorded on a JEM-2100 transmission electron microscope (TEM, JEOL, Japan) at 200 kV. Samples were suspended in ethanol, fully dispersed by ultrasonic wave, and deposited on an amorphous carbon coated copper grid prior to observation. The JEM-2100 TEM equipped with EDX spectrometry was also used for the energy-dispersive

X-ray (EDX) analysis and selected area electron diffraction (SAED). Powder X-ray diffraction (XRD) patterns were collected using a D/max-2200/PC X-ray diffractometer (Rigaku, Japan) fitted with nickel-filtered Cu Kα radiation. The data were collected at 0.02° intervals with counting for 0.2 s at each step. X-ray photoelectron spectroscopy (XPS) was conducted using an ESCALAB 250 X-ray photoelectron spectrometer (Thermo Scientific, US) with non-monochromatic Al K_α X-ray (1486.6 eV). The analyzer was operated at 20 eV pass energy with an energy step size of 1 eV (full spectra) and 0.1 eV (high-resolution spectra). Binding energy calibration was based on C 1*s* at 284.6 eV. The size distribution of the samples were determined by a Nano ZS90 particle size and zeta potential analyzer (Malvern, UK) based on dynamic light scattering (DLS) at a scattering angle of 90°. Absorption spectra were recorded by a Lambda 750S UV-Vis-NIR spectrophotometer (PerkinElmer, US), background corrected for any water contribution. NIR emission spectra were collected on a Fluorolog-3 fluorescence spectrophotometer (Horiba Jovin Yvon, France) equipped with a liquid nitrogen cooled InGaAs detector (800-1600 nm), applying an excitation laser of 488 nm. Photoluminescence lifetime was measured on the Fluorolog-3 fluorescence spectrophotometer at 25 °C. The sample was excited by the 488 nm laser with a pulse duration of 5 μs. The fluorescence decay data were fitted using a biexponential model. The Ag concentration in different parts of the mice were determined on an X series inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Elemental, UK).

Determining photoluminescence quantum yield of Ag_2Se QDs

The quantum yield of as-synthesized Ag_2Se QDs was measured using indocyanine green (ICG) as a reference (QY = 13% in DMSO). The absorption spectra of the Ag_2Se QDs and ICG solutions

at different concentrations were recorded. Then the fluorescence spectra of these samples were recorded under the same conditions. The photoluminescence quantum yield was calculated according to the following equation:

$$\phi_{QD} = \phi_{ICG} * \left(\frac{F_{QD}}{F_{ICG}} \right) * \left(\frac{A_{ICG}}{A_{QD}} \right) * \left(\frac{n_{QD}}{n_{ICG}} \right)^2 \quad (1)$$

Where ϕ_{QD}, F_{QD}, A_{QD} and n_{QD} are the quantum yield, integrated fluorescence intensity, integrated absorption and refractive index of the solvent for the Ag_2Se QDs. The parameters with a subscript of ICG are corresponding quantities of the solvent for ICG.

Cytotoxicity assay

HeLa cells were cultured in DMEM supplemented with 10 wt% FBS, 100 IU/mL penicillin and 100 μg·mL^{-1} streptomycin in a humidified incubator with 5 vol% carbon dioxide at 37 °C. L929 cells were cultured in RPMI 1640 medium supplemented with 10 wt% FBS, 100 IU/mL penicillin and 100 μg·mL^{-1} streptomycin in the humidified incubator under the same conditions. The media were refreshed every 2 or 3 days according to cell density.

Cytotoxicity of the MDP-capping Ag_2Se QDs was evaluated by trypan blue assay. The L929 cells were seeded in 96-well culture plates at a density of ~ 4500 cells per well and incubated at 37 °C for 24 h for cell attachment. The culture medium in each well was then replaced by a fresh medium containing the MDP-capping Ag_2Se QDs at different concentrations (0.01 mg/mL - 1 mg/mL). One row of the 96-well plates was used as control. After further incubation for 24 h or 48 h, the culture plates were rinsed with HBSS to remove unattached cells. The remaining cells were added in HBSS to make cell suspension. 0.5 mL of 0.4 % trypan blue solution (w/v) was transferred to a test tube, in which 0.3 mL of HBSS and 0.2 mL of the cell suspension were added

and mixed thoroughly. After 10 min, a pipette was used to transfer a small amount of trypan blue-cell suspension mixture to a hemacytometer (Hausser Scientific, US). All the cells in the 1 mm center square and four 1 mm corner squares were counted. Non-viable cells would stain blue. The cell viability was obtained according to the following equation:

$$\text{cell viability \%} = \text{total viable cells (unstained)} / \text{total cells} \times 100\ \% \quad (1)$$

Small animal imaging

Animal studies were conducted according to protocols approved by the Animal Ethics Committee of the Institute of Hematology and Hospital of Blood Diseases, Chinese Academy of Medical Sciences and Peking Union Medical College. Nude mice (18-23 g) purchased from Laboratory Animal Center of Nanjing Medical University were anesthetized with 10 % chloral hydrate via intraperitoneal injection. Then 10 μg of MDP-capping Ag_2Se QDs (0.1 mg/mL in PBS) were injected into tail vein of the nude mice. After 12 h, the mice were positioned on an imaging platform, and NIR fluorescence images were recorded using the liquid-nitrogen-cooled InGaAs camera by collecting photons in NIR-II region. The excitation source was an 808 nm laser diode coupled to a 4.5 mm focal length collimator. The emission from the animal was filtered through a 900 nm long-pass filter coupled with the InGaAs camera. The excitation power density at the imaging platform was 140 mW/cm^2.

Supplementary data

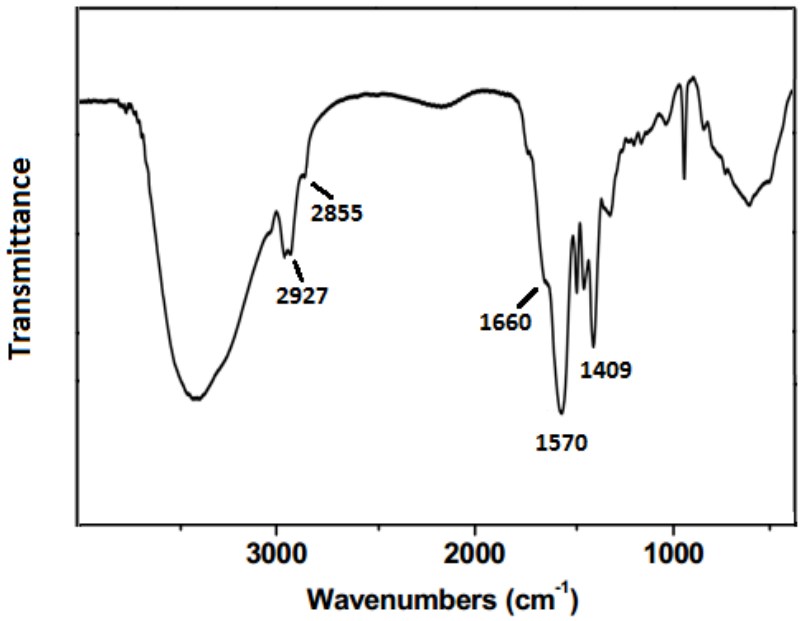

Figure. 1S FTIR spectrum of MDP-capping Ag_2Se QDs. The peaks at 1570 and 1409 cm^{-1} are ascribed to -COO-. The peaks at 2927 and 2855 cm^{-1} are assigned to -CH_2 in the -CH_2 chain from the PAA backbone. The absorption at 1660 cm^{-1} corresponds to the -CONH- from the PAA-*g*-MEA ligand.

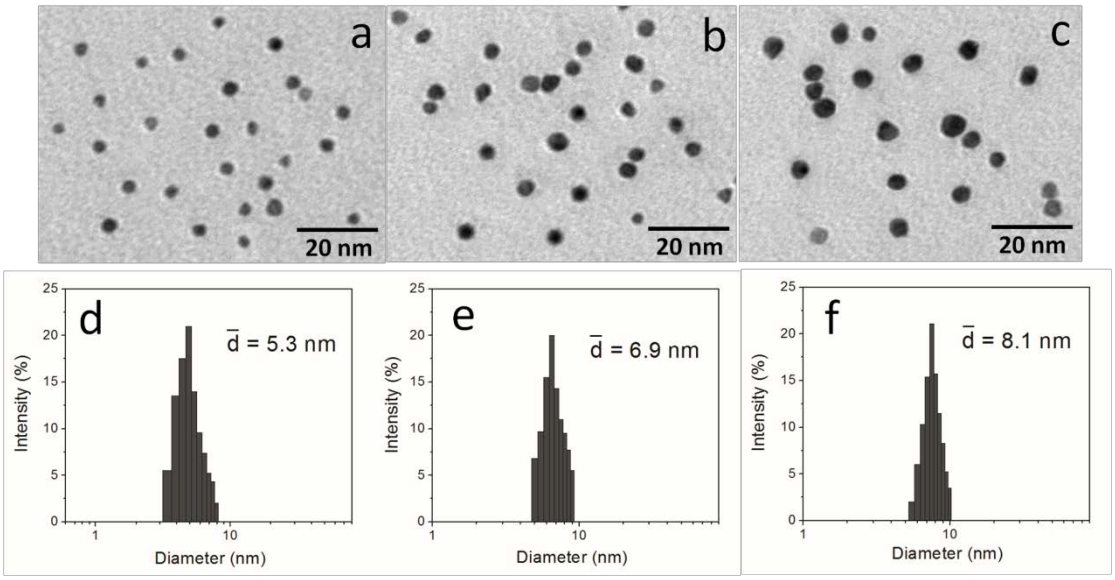

Figure. 2S TEM micrographs (a-c) and the corresponding size distribution based on DLS (d-f) of MDP-capping Ag_2Se QDs synthesized over varied reaction times: (a,d) 0.5 h; (b,e) 1.5 h; (c,f) 3 h.

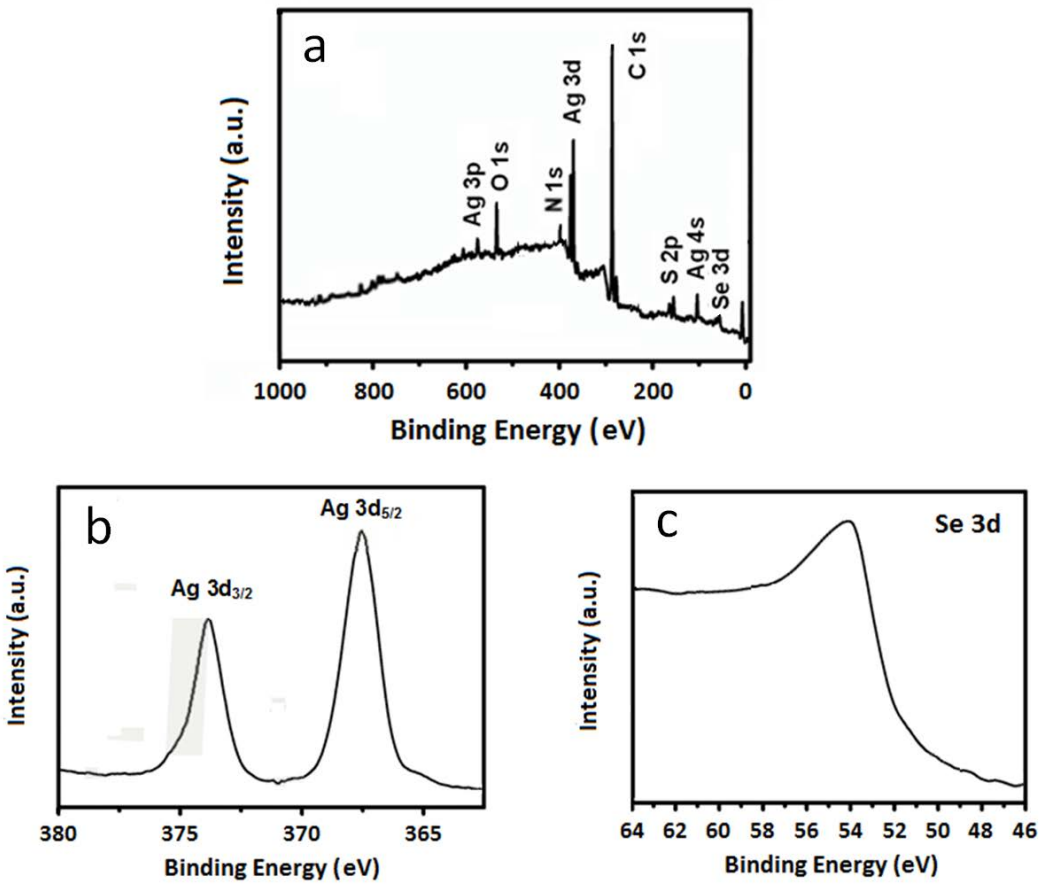

Figure. 3S (a) Full XPS spectrum of MDP-capping Ag_2Se QDs. (b) Ag 3*d* signals and (c) Se 3*d* signals recorded for the Ag_2Se QDs. Valence state: Se, -2; S, -2.

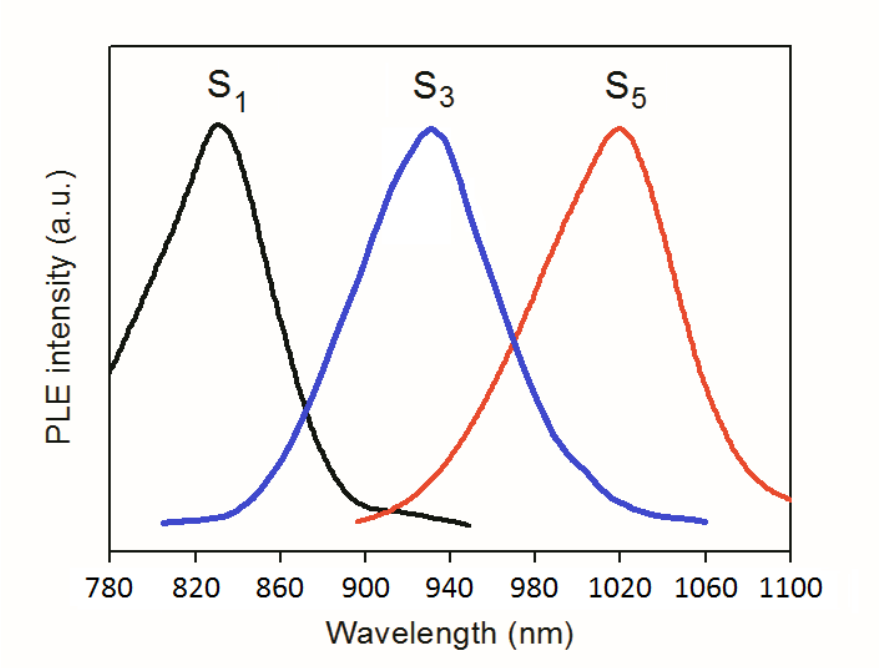

Figure. 4S PL excitation spectra of three different Ag_2Se QDs samples in NIR-I region.

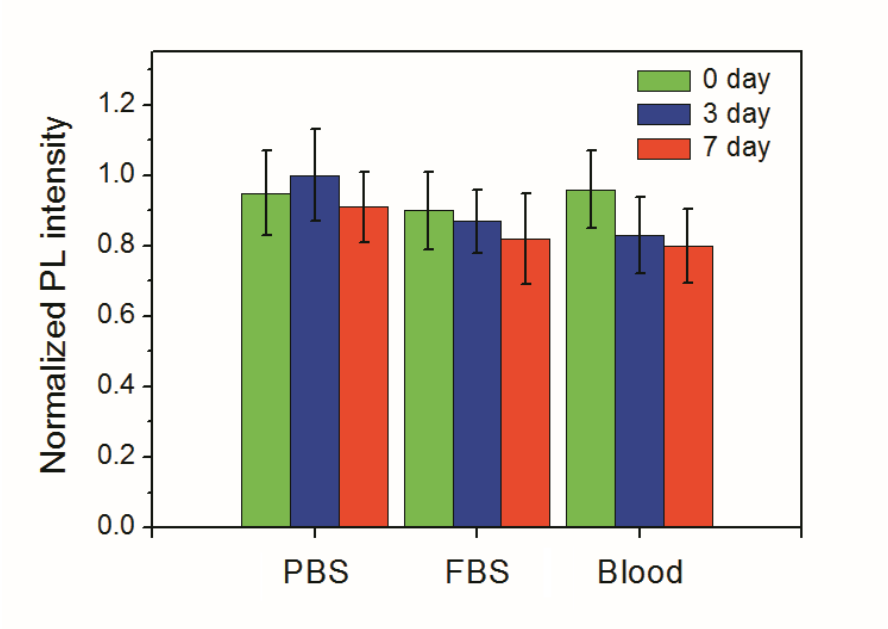

Fig. 5S PL intensity changes of MDP-capping Ag_2Se QDs stored in different media at 37 °C during a period of 7 days.

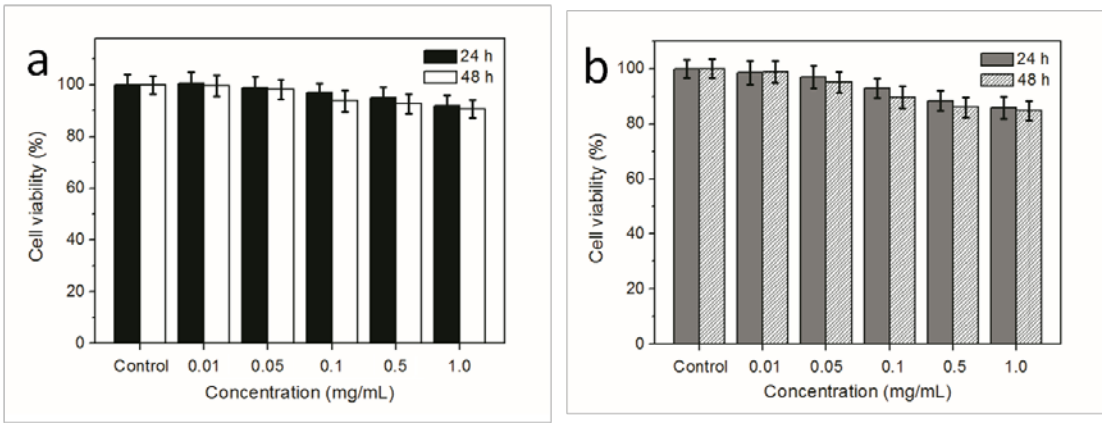

Figure. 6S Viability of (a) HeLa cells and (b) L929 cells incubated with MDP-capping Ag_2Se QDs for different times based on trypan blue exclusion assay.