Supporting Information

A facile cation exchange-based aqueous synthesis of highly stable and biocompatible Ag_2S quantum dots emitting in the second near-infrared biological window

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[1] *These authors have the equivalent contribution to this work.*

1. Apparatus

The photoluminescence (PL) lifetime studies were performed using an Edinburgh FL nF900 mode single-photon counting system equipped with a Hydrogen lamp as the excitation resource (Edinburgh Instruments, U.K.). The energy diffraction X-ray (EDX) spectra and the selected area electron diffraction (SAED) images were obtained with a JEOL JEM-1400 transmission electron microscope (TEM, JEOL, Japan) at an acceleration voltage of 120 kV. The powder X-ray diffraction (XRD) patterns were obtained with wide-angle X-ray scattering, using a D5005 X-ray powder diffractometer that equipped with graphite monochromatized Cu Kα radiation (λ = 1.54056Å) (Siemens, Germany). The inductively coupled plasma atomic emission spectroscopy (ICP-AES, Integra XL, Australia) using a standard HCl/HNO_3 digestion was utilized to measure the concentrations of Cd ions.

2. Results

Table S1 The exchange efficiency of Cd ions (from CdS QDs) with Ag ions.

Samples	PL emission/nm	[a] $[Cd]_{before}$/μg mL^{-1}	[b] $[Cd]_{after}$/μg mL^{-1}	[c] EE/%	[d] RSD/%
CdS (*A*)	526	49.94	0.12	99.76	3.09
CdS (*B*)	554	50.85	0.06	99.88	0.52
CdS (*C*)	575	50.53	0.24	99.52	3.56
CdS (*D*)	589	49.87	0.17	99.66	4.16

[a, b] The concentrations of Cd ions in CdS QDs water-dispersible solution before and after cation exchange between Cd and Ag ions due to the addition of $AgNO_3$ (the mol ratio of Ag/Cd is more than 2/1, *i.e.*, slightly excessive Ag) solution. All concentrations were measured by ICP-AES, and expressed as means of six repeated measurements.

[c] The cation exchange efficiency (EE, %) = 100 × ($[Cd]_{before}$ - $[Cd]_{after}$) / $[Cd]_{before}$.

[d] The relative standard deviation (RSD, %) of EE was defined as 100 × (relative standard) / mean.

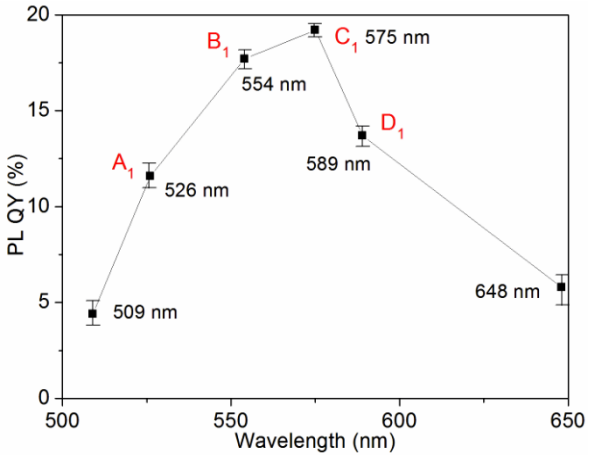

Fig. S1 PL QYs of CdS QDs prepared at different time points from 10, 30 min, 1, 2, 3 to 6 h, corresponding to the maximum emission peak wavelengths from 509, 526 (A_1), 554 (B_1), 575 (C_1), 589 (D_1) to 648 nm. The QYs were calculated by the equation: $\Phi_s = \Phi_r (I_s/I_r) (A_r/A_s) (n_s/n_r)$, where the Φ_s, I_s, A_s and n_s are QYs, emission peak area, integrated absorption intensity and refractive indices of QDs, respectively, and the Φ_r, I_r, A_r and n_r stand for the corresponding parameters of rhodamine 6G as a reference standard (QYs = 94% in water).

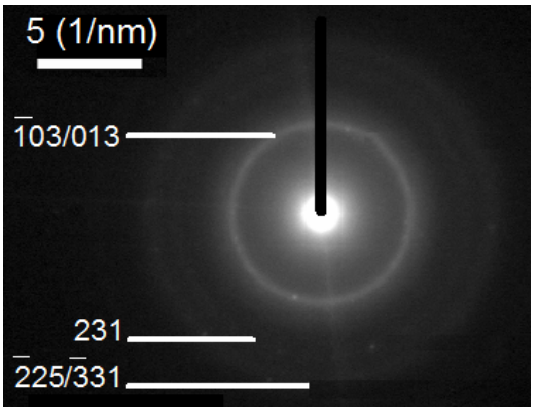

Fig. S2 The selected area electron diffraction (SAED) pattern of Ag_2S QDs (the sample D_2) prepared from CdS QDs (the sample D_1) *via* cation exchange.

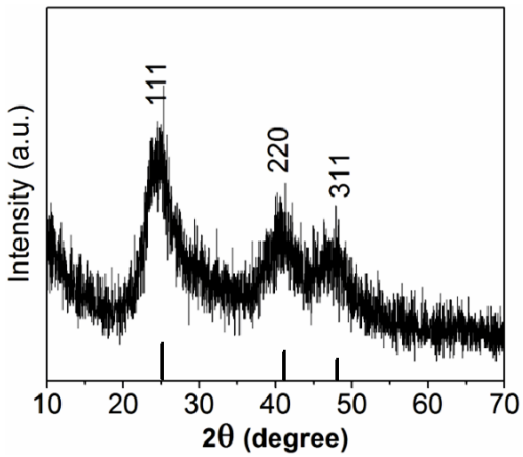

Fig. S3 Powder XRD patterns of the initial CdS QDs (the sample D_1) prepared in this study based on a facile aqueous synthesis, and corresponding diffraction peak positions of cubic zinc blende CdS bulk crystals (JCPDS Card No. 10-454).

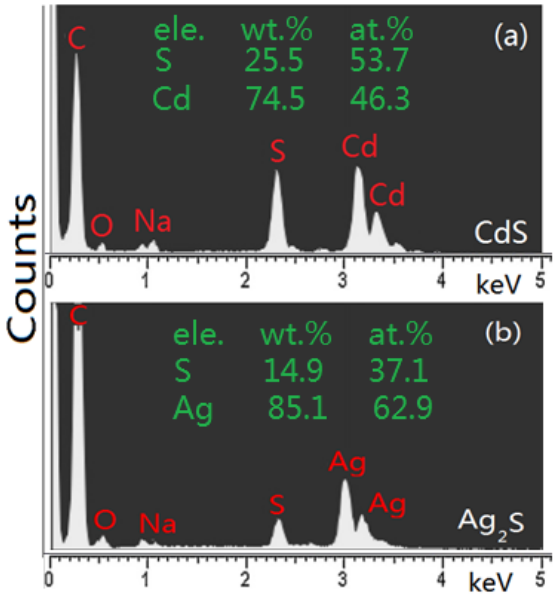

Fig. S4 EDX spectra of (a) CdS QDs (the sample D_1) and (b) Ag_2S QDs (the sample D_2) prepared from CdS QDs *via* cation exchange (or CdS QDs after the addition of Ag^+ ions).

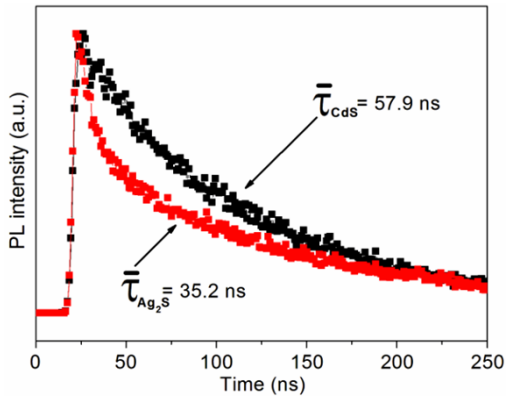

Fig. S5 PL decay curves and average PL lifetimes of CdS QDs (the sample D_1) and Ag_2S QDs (the sample D_2) prepared from CdS QDs *via* cation exchange. Here, the average PL lifetime means the room temperature PL decay time (*i.e.*, the exciton lifetime, $\tau_{1/e}$), at which the PL intensity has decreased to 1/e of its initial value, is used as a parameter to compare the lifetime.

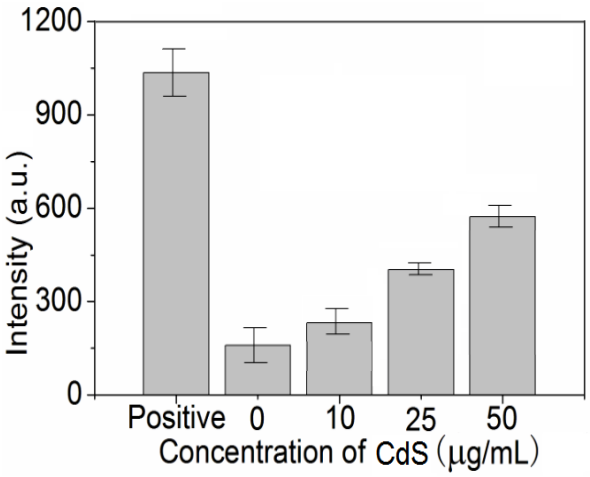

Fig. S6 Quantitative flow cytometry results. ROS induced by CdS QDs (0, 10, 25, and 50 μg mL^{-1}) after 72 h treatment. Positive represents the positive control with Rosup concentration of 50 μg mL^{-1}.

3. NIR-II PL cell *in situ* imaging

The performance of Ag_2S QDs as NIR-II PL cell imaging was estimated as below. Briefly, a portion of L929 cell suspension (10 mL) was transferred to 200 mL of PBS (1 mM, pH 7.4), and the resulting mixture was placed into a 6-well chamber slide, storing in a temperature-controlled chamber (at 37 °C for 24 h, 1 L min^{-1} of CO_2 gas flow). Then, Ag_2S QDs dispersed in water (50 μg mL^{-1}) were added into the cell dishes. After incubating for 2 h at 37 °C, the QDs-loaded cells were washed three times with PBS to remove free QDs absorbed or/and attached on the outer surface of cell membrane. The cell imaging was done using a laser diode (658 nm excitation) with 80 μm diameter spot focused by a 100× objective lens. The NIR-II PL was collected by a liquid-nitrogen-cooled, 320 × 256 pixel, and two-dimension lnGaAs camera with a sensitivity range from 800 to 1700 nm. The excitation light was filtered out using a 900 nm long-pass filter so that the intensity of each pixel represented light in 900~1700 nm. The NIR-II PL images were taken at 300 ms of exposure time. For the bright field images, a fiber optic illuminator was used for illuminating the sample in the transillumination mode, and the images were taken using the same filter at 2 ms of exposure time. Fluorescence imaging of tumor cells was recorded on a FV-300 IX 71 Confocal Fluorescence Microscope (CFM, Olympus).

To illustrate the feasibility of highly stable and biocompatible Ag_2S QDs as effective NIR-II PL emissive probes, L929 cell imaging was performed in the range of 900~1700 nm using the intrinsic NIR-II PL of Ag_2S QDs (directly obtained from CdS QDs *via* cation exchange). As shown in **Fig. S7**, the cell line treated with Ag_2S QDs exhibited bright NIR-II PL signal (typical red-white colour).[1,2] The Ag_2S QDs showed a punctuated cytoplasmic distribution, indicative of confinement in endosomes and lysosomes (subcellular locations) as common for QDs. These observed results indicated that the QDs can be used as a new type of NIR-II-emitting nanoprobe with high colloidal stability, favorable photostability, bright PL emission, as well as outstanding biocompatibility and ultralow cytotoxicity.

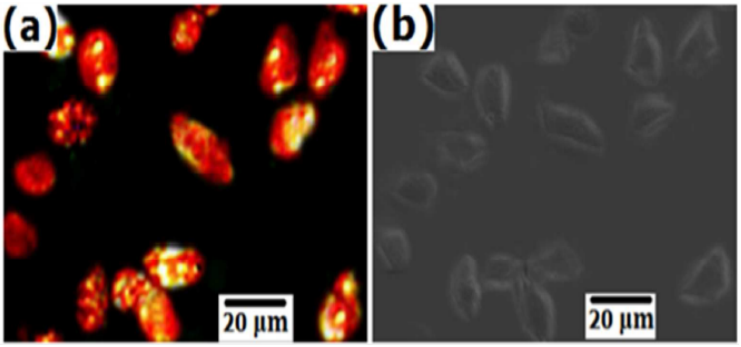

Fig. S7 (a) NIR-II PL image (900~1700 nm) and (b) corresponding optical image in vitro of L929 cells treated with Ag_2S QDs (50 μg mL^{-1}).

References

(1) Zhang, Y.; Hong, G.; Zhang, Y.; Chen, G.; Li, F.; Dai, H.; et al. Ag_2S Quantum dot: A bright and biocompatible fluorescent nanoprobe in the second near-infrared window. *ACS Nano* **2012**, *6*, 3695-3702.

(2) Hong, G.; Robinson, J. T.; Zhang, Y.; Diao, S.; Antaris, A. L.; Wang, Q.; Dai, H. In-vivo fluorescence imaging with Ag_2S quantum dots in the second near-infrared region. *Angew. Chem. Int. Ed.* **2012**, *51*, 9818-9821.