Supplementary Information

Energy Transfer Limitation between NIR-II PEGylated Quantum Dots and Water

Mingzhe Wang,[a,b] Sisi Ling,[b] Ziyan Zhang,[a, b] Yejun Zhang,[b]* Hongchao Yang[b] and Qiangbin Wang[a,b]*

[a] School of Nano-Tech and Nano-Bionics, University of Science and Technology of China, Hefei 230026, China

[b] CAS Key Laboratory of Nano-Bio Interface, Division of Nanobiomedicine and *i*-Lab, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Suzhou 215123, China.

Email: yjzhang2009@sinano.ac.cn; qbwang2008@sinano.ac.cn

Experimental Section

Materials

1-Dodecanethiol (DT, 98%), dimethyl sulfoxide (DMSO) and Oleylamine (OAm, 80%) were purchased from Acros. Sliver nitrate ($AgNO_3$, 99.8%), $(C_2H_5)_2NCS_2Na{\cdot}3H_2O$ [Na(DDTC), 99.0%], chloroform (99.0%) were obtained from Sinopharm Chemical Reagent Company. Oleic acid (OA, 90%), 11-mercaptoundecanoic acid (MUA, 95%), deuteroxide (D_2O), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC), N-Hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. Lead (II) chloride ($PbCl_2$, metals basis, 99.999%) was purchased from Alfa Aesar. Methoxy Polyethylene Glycol Amine (PEG-NH_2), methoxy polyethylene glycol thiol (PEG-SH) was purchased from Xi'an Ruixi Biolggical Technology. All these reagents were of analytical grade and used without further purification.

Synthesis of Ag_2S-PEG QDs

DT-encapsulated Ag_2S QDs (Ag_2S-DT) were obtained using thermal decomposition of Ag(DDTC). 0.2 mmol Ag(DDTC) was added to 20 g DT in a three-neck flask (100 mL) at room temperature. The flask was isolated from oxygen and stirred in vacuum with strong magnetic force for 15 min. The reaction temperature was then increased to 200 °C at a rate of 10 °C/min and kept at this temperature for 45 min to allow the Ag_2S QDs to grow under N_2 atmosphere. The Ag_2S-DT QDs were obtained at the end of the reaction, purified with ethanol and freely dispersed in chloroform solution. Ag_2S-PEG QDs were prepared using a ligand exchange strategy. First, 2 mg of Ag_2S-DT QDs were dispersed in 10 mL of chloroform. Then 60 mg of HS-PEG (2K) was added to the above solution and stirred for 24 h. The solution was purified with hexane, centrifuged, and the precipitate was dispersed in equal amounts of water and chloroform.

Synthesis of PbS-PEG QDs

Synthesis of PbS nanocrystals: In a typical synthesis of PbS nanocrystals (NCs), 2.5 mmol S (80 mg), 0.2 mmol ODA (60 mg), and 6.1 g OAm was loaded in a 100 mL three-neck flask and heated to 120 °C under nitrogen flow. The mixture was magnetically stirred for 30 min to form an S precursor solution (OAm-S). Then, the temperature of OAm-S was decreased to 80 °C for later use. 0.15 mmol $PbCl_2$ (0.417 g) and 6.1 g OAm were loaded into another 100 mL three-neck flask and heated to 120 °C under nitrogen flow and magnetically stirred for 30 min to obtain a Pb precursor solution. 2.25 mL OAm-S were injected into the Pb precursor solution. After a 2 min of reaction period, the heat source was removed, and 10 mL hexane was immediately injected into the mixture to stop the reaction. The solution was centrifuged (3500 rpm, 5 min) to remove excess $PbCl_2$. 5 mL OA was added to the supernatant and violently oscillated to replace the capping ligand of PbS NCs to OA. Finally, the products were purified with ethanol by centrifugation (10000 rpm, 3 min) and redispersed in 10 mL chloroform. PbS-PEG QDs were prepared using a ligand exchange strategy. 3 mg of PbS NCs were dispersed in 10 mL of chloroform. Then 60 mg of HS-PEG (2K) was added to the above solution and stirred for 24 h. The solution was purified with hexane, centrifuged, and the precipitate was dispersed in equal amounts of water and chloroform.

Synthesis of Ag_2S-MUA QDs

MUA-capped Ag_2S (Ag_2S-MUA) QDs were prepared using a facile ligand exchange strategy. First, 2 mg Ag_2S-DT QDs were dissolved in 10 mL of chloroform. Then, 0.2 g of MUA was added to the above solution with stirring for 24 h. Subsequently, it was centrifuged, and the precipitate was dispersed in 2 mL water with pH of 9.0 adjusted by $NH_3 \cdot H_2O$.

Synthesis of Ag_2S-C_{11}-PEG QDs

18 mg of MUA with 200 mg of NH_2-PEG was added to 3 mL of chloroform in a glass sample vial at room temperature, then 143 mg of NHS and 240 mg of EDC were added to a quantity of DMSO, and shaken to mix well and then added to a bobbin sample vial. The reaction was stirred with strong magnetic force at room temperature for 12 h. At the end of the reaction, 3 mg of Ag_2S-DT was added for ligand exchange, after which it was purified with hexane, centrifuged, and the precipitate was dispersed in water.

Absorption, photoluminescence, photoluminescence lifetime measurements and structure characterization

Absorption spectra were recorded with a Biomate 160 UV-vis-NIR spectrophotometer (ThermoFisher Scientific, USA). NIR photoluminescence spectra were executed on a near IR Spectrometer (ideaoptics) at room temperature, applying an excitation laser source of 808 nm. The PL time decays were recorded on was measured with a calibrated spectrofluorometer (Edinburgh Instruments, FLS1000) equipped with an integrating sphere. A laser with 800 nm was used as the excitation source. The morphologies of the products were examined through a Tecnai G2 F20 S-Twin transmission electron microscope (TEM) at an acceleration of 200 kV.

Transient absorption measurements

TAS measurements were performed on a Helios (Ultrafast Systems) spectrometer using a regeneratively amplified femtosecond Ti: sapphire laser system (Spitfire Pro-F1KXP, Spectra-

Physics; frequency, 1 kHz; max pulse energy, ~8 mJ; pulse width, 120 fs) at room temperature. Finally, analyze the data through commercial software (Surface Xplorer, Ultrafast Systems).

Energy Transfer Efficiency Measurement

According to the PL lifetime of the QDs, the ET process in Ag_2S-C_{11}-PEG QDs is effectively suppressed. Consequently, the intensity of ET (I_{ET}) can be represented by the difference in PL intensity between Ag_2S-PEG (I_W) and Ag_2S-C_{11}-PEG QDs ($I_{W, C11}$) in water.

$$I_{ET} = I_{W, C11} - I_W \quad (1)$$

Thus, the efficiency of ET (η_{ET}) to the fluorescence quenching can be described by Eq2, where the I_C denotes the PL intensities of Ag_2S-PEG QDs in chloroform.

$$\eta_{ET} = I_{ET}/I_C \times 100\% = (I_{W, C11} - I_W)/I_C \times 100\% \quad (2)$$

Photothermal Efficiency Measurement

A 1064 nm laser with 1 W/cm^2 was used to irradiate the sample through a transparent sample plate. Chromatography thermocouple was inserted into sample cell, the other end connected to digital thermometer. After resonant irradiation to the samples (Ag_2S-PEG and Ag_2S-C_{11}-PEG in water and pure water) with 600 s, the laser was discontinued and the sample was allowed to cool, returning to a value in equilibrium with its surroundings.

MTT Assay

L929 cells were seeded in a 96-well plate at 1×10^4 cells per well and incubated with different concentration of Ag_2S-PEG and Ag_2S-C_{11}-PEG QDs for 48 h, respectively. The different concentrations were 0, 20, 40, 80, 100, 200 μg/mL. A stock solution of MTT (20 μL, containing 0.1 mg) dissolved in PBS was added, followed by another 4 h incubation. The media was removed and 100 μL of DMSO was added to dissolve the MTT-formazan generated by cells. Absorbance at 550 nm of each well was measured using an enzyme-linked immunosorbent assay (ELISA) reader (infinite M200, Tecan, Austria). The cell growth viability was calculated through the formula shown below:

Cell Viability (%) = (mean of absorbance value of treatment group /mean absorbance value of control) ×100

Animal models

Animal procedures studies and procedures were performed under Chinese Laboratory Animal Management Regulations protocols. All animal experiments were approved by the Animal Care and Use Committee of the Institutional Animal Committee of the Chinese Academy of Sciences (assigned approval number: SINANO/EC/2022-008).

In vivo NIR imaging

Mice were intravenous injected with Ag_2S-PEG and Ag_2S-C_{11}-PEG at the dosage of 5 mg kg^{-1}, respectively. Time course of NIR imaging were carried out with a DaVinci YR-1000 In Vivo Imaging System (Suzhou NIR-Optics Co., Ltd., China) (exposure time=300 ms).

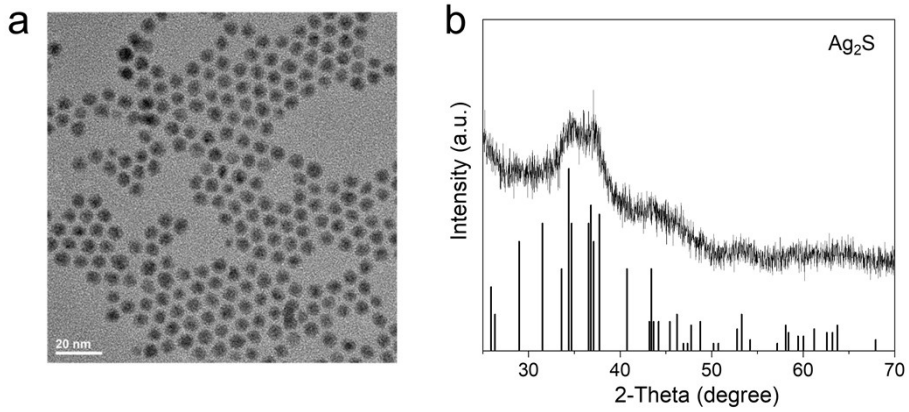

Fig. S1. (a) TEM image and (b) X-ray diffraction (XRD) pattern of Ag_2S QDs.

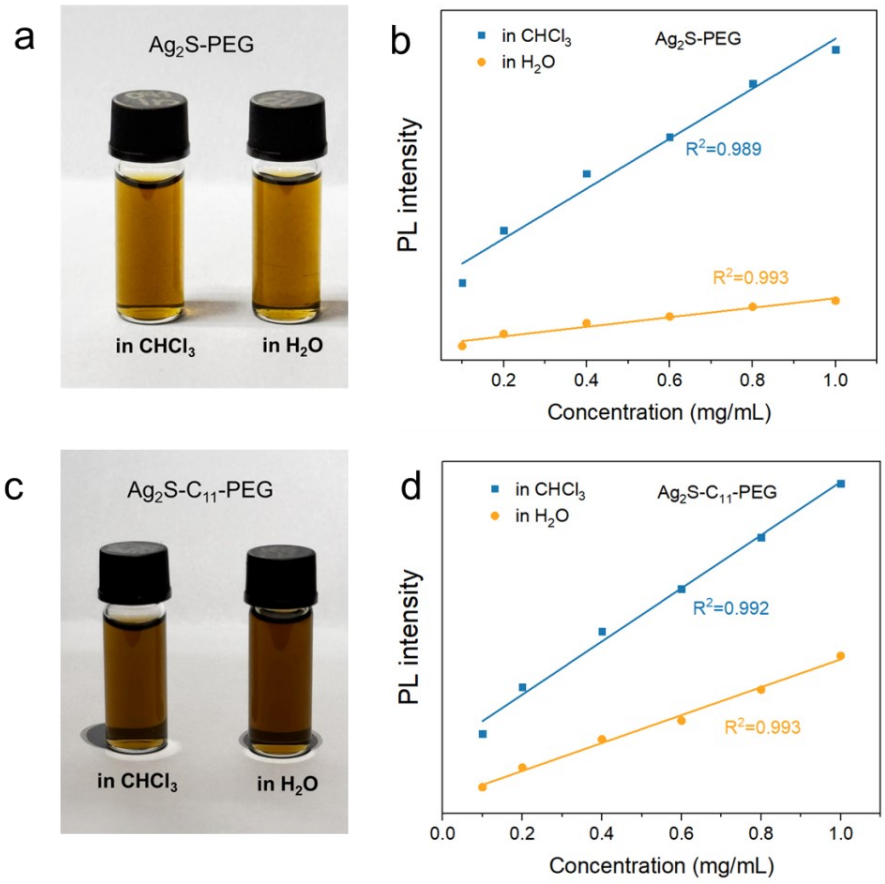

Fig. S2. The digital photographs of (a) Ag_2S-PEG and (c) Ag_2S-C_{11}-PEG QDs left for 4 months. The QD-concentration-dependent PL intensity of (b) Ag_2S-PEG QDs and (d) Ag_2S-C_{11}-PEG QDs disperse in water and chloroform.

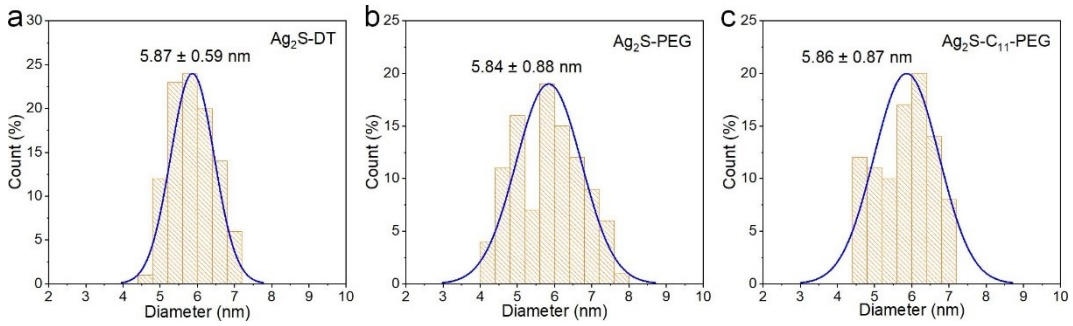

Fig. S3. Size distribution diagram of (a) Ag_2S-DT, (b) Ag_2S-PEG and (c) Ag_2S-C_{11}-PEG QDs.

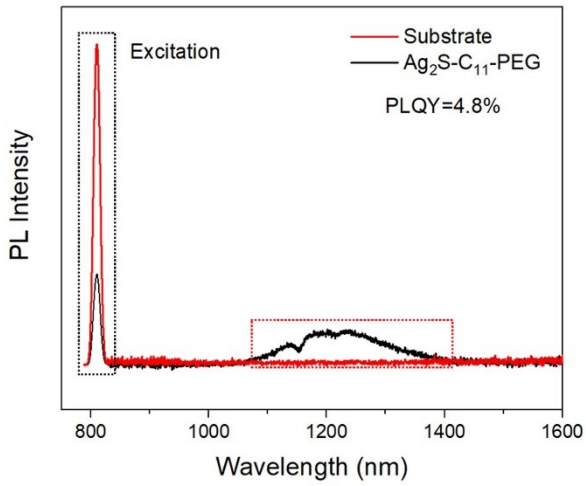

Fig. S4. Absolute PLQY of Ag_2S-C_{11}-PEG QDs.

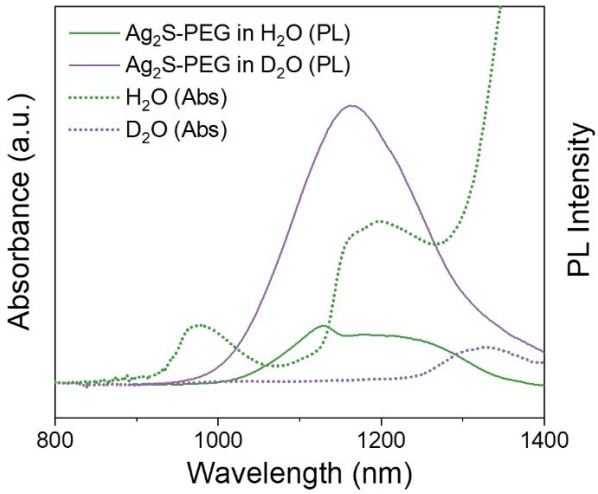

Fig. S5. Absorption and PL spectra of the Ag_2S-PEG QDs dispersed in H_2O and D_2O.

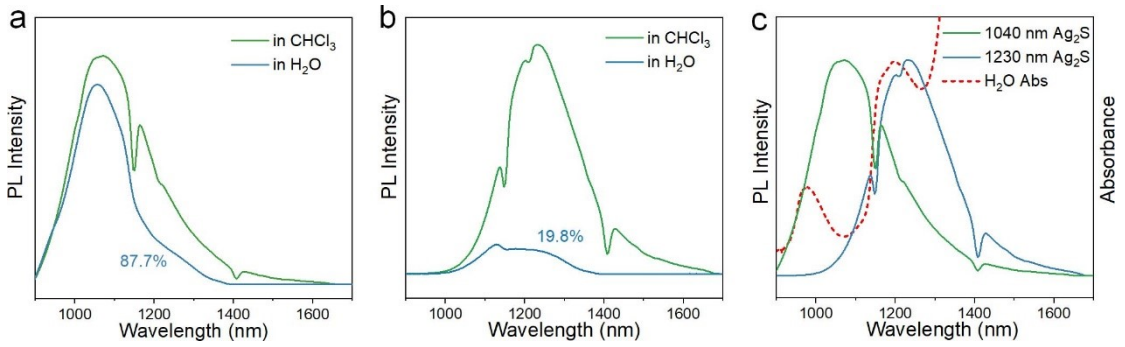

Fig. S6. PL spectra of (a) Ag_2S-PEG QDs (1040 nm) and (b) Ag_2S-PEG QDs (1230 nm) in chloroform and water. (c) Relative spectra overlap of Ag_2S-PEG QDs emission with diverse bandgap and H_2O absorption.

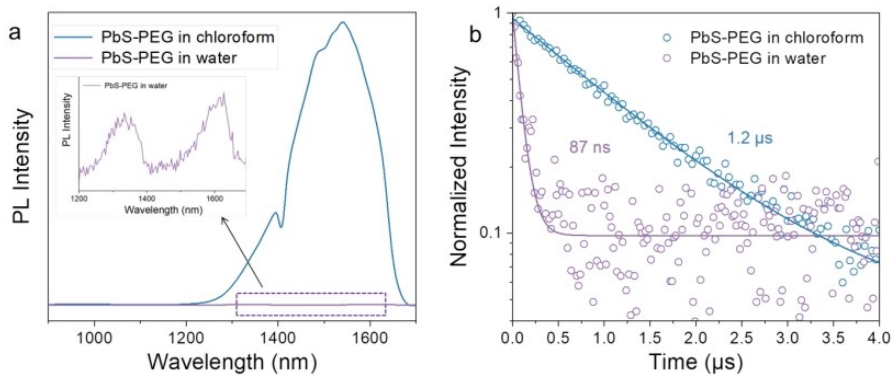

Fig. S7. (a) PL spectra and (b) time-resolved PL decay of PbS-PEG QDs dispersed in chloroform and water.

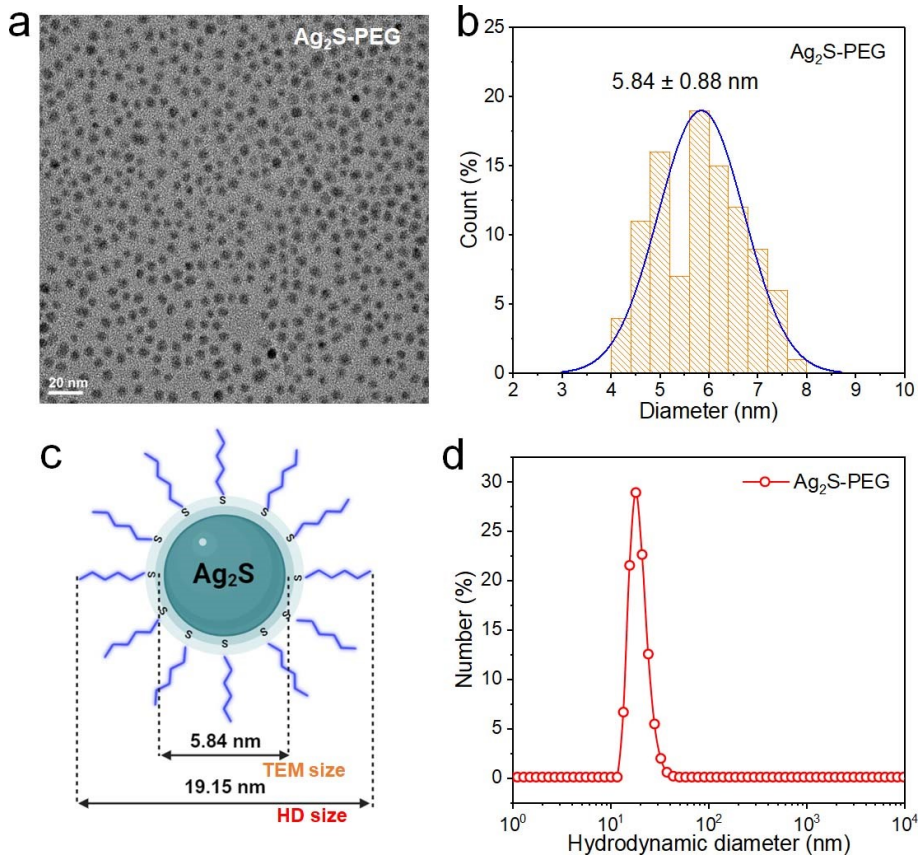

Fig. S8. (a) TEM image, (b) size distribution diagram from TEM, (c) the description of QD size and (d) the hydrodynamic diameter of Ag_2S-PEG QDs.

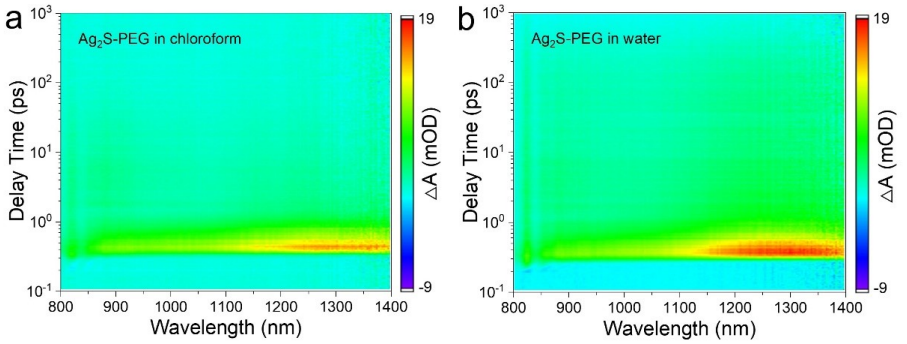

Fig. S9. Two-dimensional pseudocolor map of TA spectra of Ag_2S-PEG in (a) chloroform and (b) water with a pump wavelength of 400 nm.

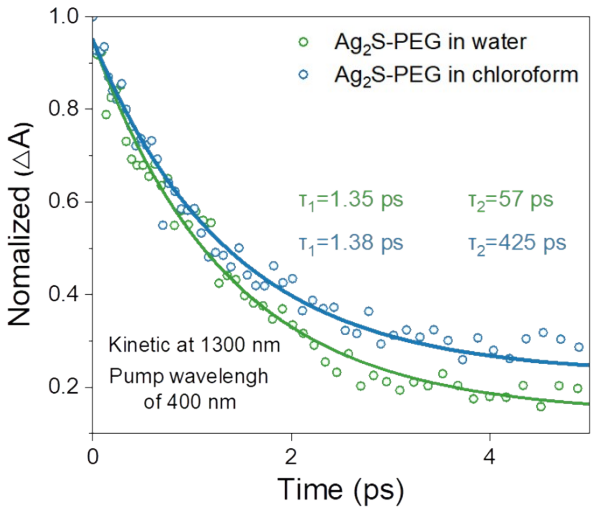

Fig. S10. Exciton decay kinetics and fits of Ag_2S-PEG observed at 1300 nm.

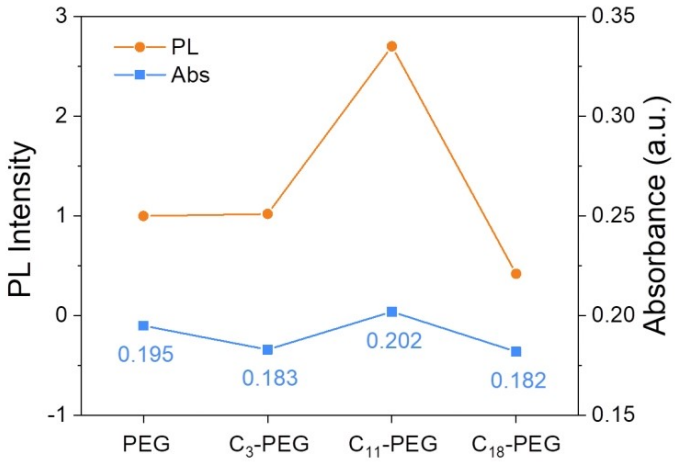

Fig. S11. Relative PL intensity fluctuation and absorbance of Ag_2S QDs with different ligands in water.

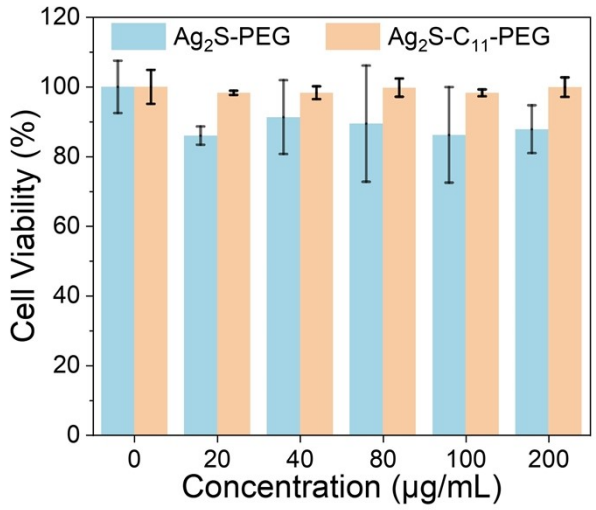

Fig. S12. MTT assay of L929 cells after treated with different concentration of Ag_2S-C_{11}-PEG and Ag_2S-PEG, respectively.

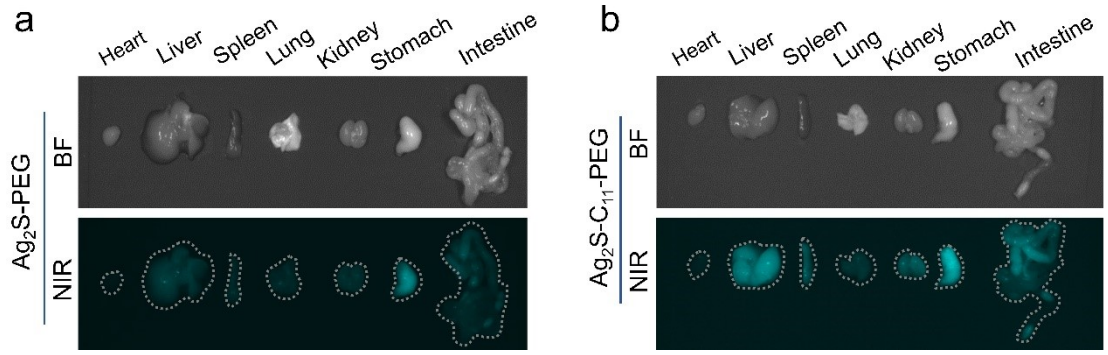

Fig. S13. NIR-II images of dissected mouse organs with intravenous injection of Ag_2S-PEG (a) and Ag_2S-C_{11}-PEG QDs (b).

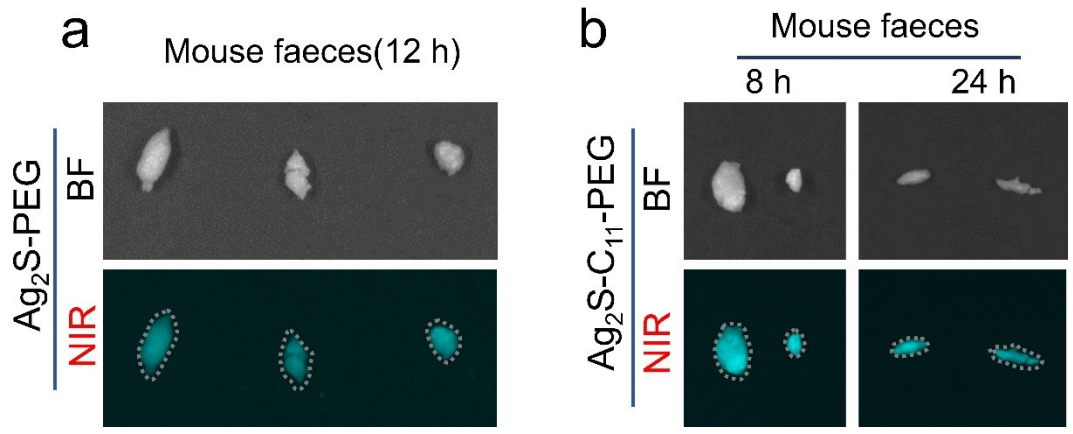

Fig. S14. NIR-II images of mouse faeces with intravenous injection of Ag_2S-PEG (a) and Ag_2S-C_{11}-PEG QDs (b).