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Doping Ag_2S Quantum Dots with Pb yields a significantly enhanced in vivo NIR-II fluorescence imaging and comparable toxic effects

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Synthesis of PEG-modified Ag_2S QDs and Pb-doped Ag_2S QDs

Ag_2S QDs and Pb-doped Ag_2S QDs were firstly synthesized in organic phase and then were transferred into hydrophilic via coating of the amphiphilic polymer, octylamine-modified poly-acrylic acid (OPA), designated as OPA-coated QDs. At first, the ODE (4 ml) was added into a three-necked flask at the room temperature, then the oxygen and water were removed from the flask by argon flowing for 30 min under vigorous magnetic stirring. Then, the sulfur powder (12.8 mg) was added into the flask and the solution was heated to 180 ℃ and kept for half an hour. Then the solution was cooled to room temperature naturally and used as S precursor. The ODE (2.5 ml) was added into another three-neck flask with argon flowing for 30 min. Next, the AgAc (16.7 mg) and OTT (140 μL) were added into flask and the solution was heated to 180 ℃ at a heating rate of 20 ℃/min. Once reached to 180 ℃ the S precursor (500 μL) was injected rapidly into the solution and the temperature was kept in 160 ℃ for 2 min and cooling to 120 ℃ rapidly for 30 min. After reaction is finished, the solution is naturally cooled down to room temperature and washed by 45 ml ethanol for several times. Then the supernatant was discarded, and the precipitates were redispersed in tetrachloroethylene and washed by acetone again and dispersed in the chloroform. For the synthesis of hydrophobic Pb-doped Ag_2S QDs, $Pb(Ac)_2$ $3H_2O$ (5.7 mg) was added into above-mentioned reaction system. The rest of the procedure was the same as Ag_2S QDs. Secondly, to transfer Ag_2S and Pb-doped Ag_2S QDs into hydrophilic, the QDs were then coated with the amphiphilic polymer, octylamine-modified poly-acrylic acid (OPA). Firstly, 30 mg of OPA were added into 20 mL of chloroform containing 10 mg of QDs. Next, the chloroform was removed using a rotary evaporator and the mixture was dispersed in borate buffer (0.05 M, pH=12), designated as OPA-Ag_2S QDs and Pb-doped Ag_2S QDs. After purification by centrifugal filter devices (Millipore Amicon,Ultra-15/40 kDa, Millipore, MA, USA) several times and size exclusion chromatography (Superdex 200 prep grade, GE Healthcare, Boston, MA, USA), the OPA-QDs were dispersed in borate buffer (0.05 M, pH=7).

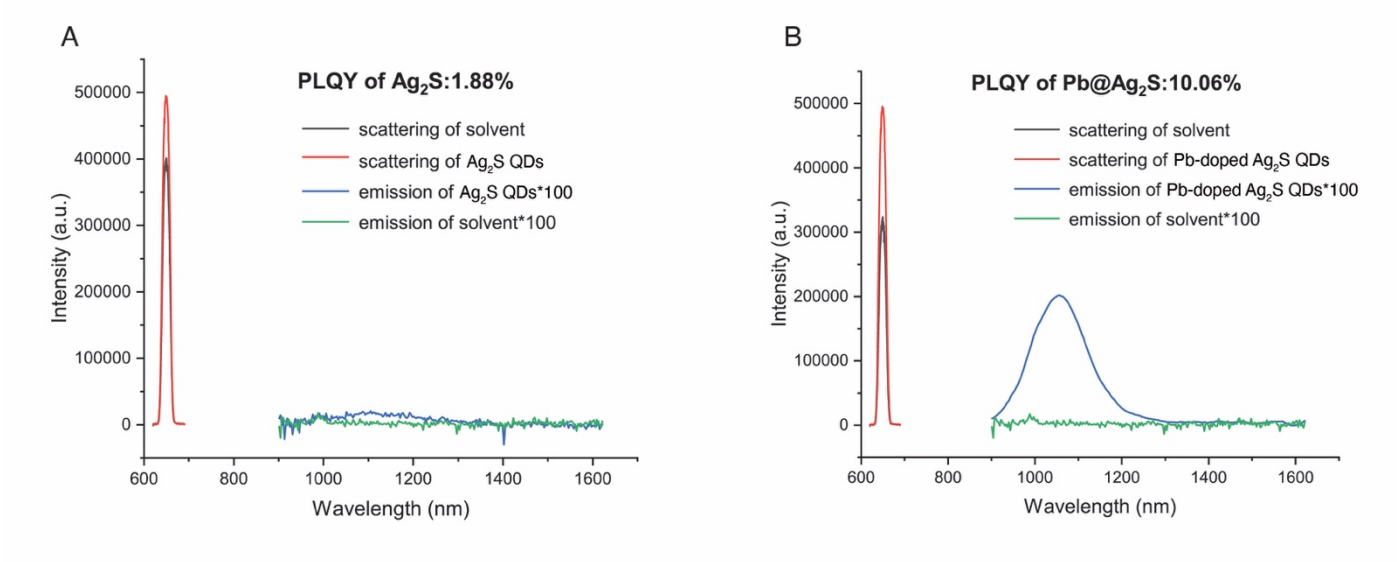

Figure S1 Absolute photoluminescence quantum yield test of Ag_2S QDs **(A)** and Pb-doped Ag_2S QDs **(B)**.

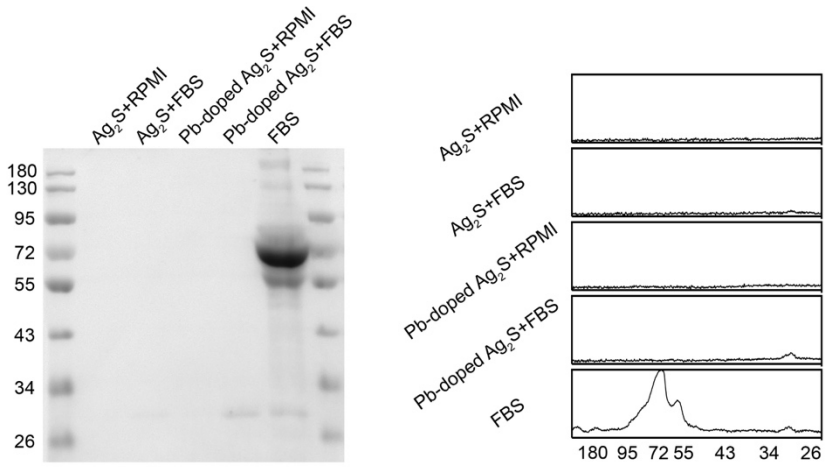

Figure S2 "Hard" protein corona formation of PEG-modified Ag_2S QDs and Pb-doped Ag_2S QDs. The amount of "hard" protein corona of QDs, which assessed the non-specific binding effects of QDs, were illustrated by SDS-PAGE images followed by densitometry analysis. There are negligible proteins absorbed in the surface of QDs regardless of incubation with RPMI medium containing 10% FBS or pure FBS.

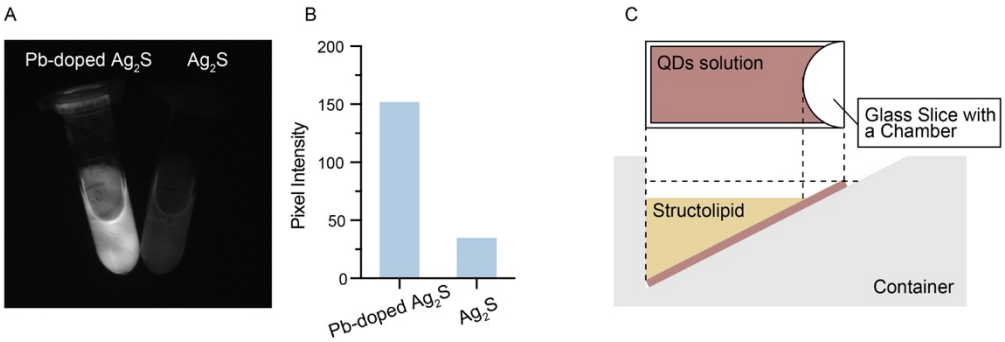

Figure S3 (A) NIR-II fluorescence images and **(B)** corresponding quantitative analysis of PEG-modified Ag_2S QDs and Pb-doped Ag_2S QDs at the identical concentration, showing an enhanced photoluminescent intensity of the latter more than 4 folds. **(C)** The schematic diagram of the *in vitro* penetration depth model.

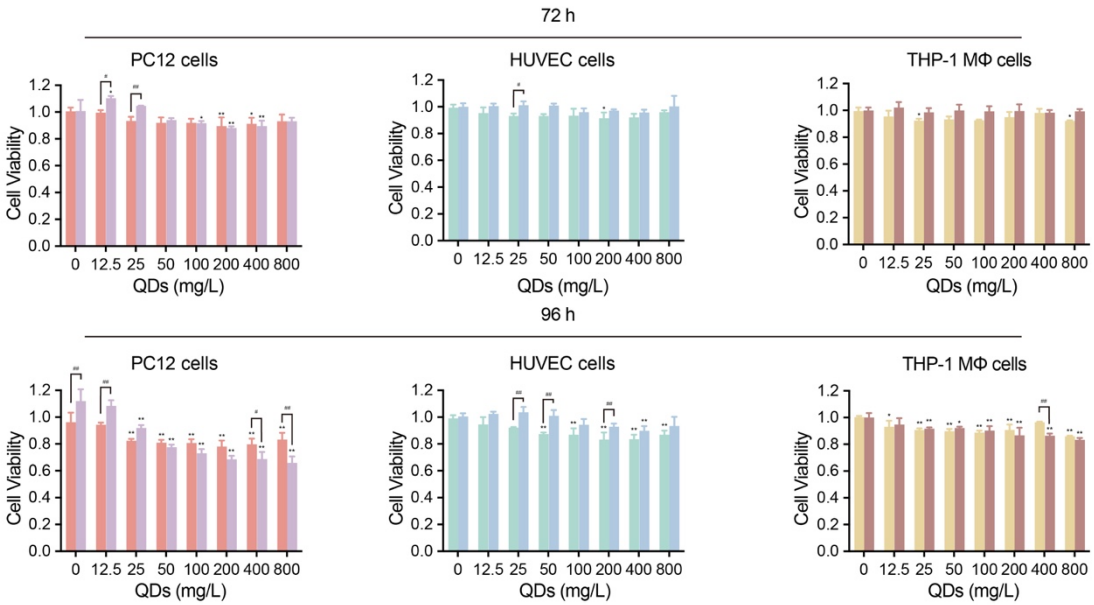

Figure S4 Continuous monitoring of cytotoxicity after removal of QDs Following exposure to QDs for 48 h, the QDs-containing medium were replaced by the normal one and cells were kept culturing for another 48 h. Then, cell viabilities of PC12 cells, HUVEC cells and THP-1 MΦ cells were measured using alamar blue assay per 24 h (total cultural time was 72 h and 96 h), which were expressed as percentage related to the untreated control cells. At 72 h, slight toxic effect was observed at the concentrations below the 800 mg/L. At 96 h, more toxic effects were observed. Above results implied adverse effects elicited by QDs may not emerged immediately. However, toxic effects triggered by two QDs were comparable during the whole cultural period. The significance of difference between the experimental groups with the control group was noted as * ($p < 0.05$) and ** ($p < 0.01$). The significance of difference between the group of PEG-modified Ag_2S QDs and that of Pb-doped Ag_2S QDs was noted as $^{\#}$ ($p < 0.05$) and $^{\#\#}$ ($p < 0.01$).