Supporting Information

Biocompatible perovskite quantum dots with superior water-resistance enable long-term monitoring of H_2S level in vivo

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Experimental section

Materials

Lead(II) bromide ($PbBr_2$, 99.999%), oleic acid (OA, 99%), oleyamine (OAm, 80-90%), ε-caprolactone (ε-CL, 99%), sodium sulfide nonahydrate (98%), chloral hydrate (>90%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, >97.0%), dimethyl sulfoxide (DMSO, >99%), paraformaldehyde (99%) and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, 98%) were purchased from Aladdin Industrial Corporation (Shanghai, China). Cesium carbonate (Cs_2CO_3, 99%) and mineral oil (Industrial grade) were obtained from Macklin Corporation (Shanghai, China). Toluene, hexane, dichloromethane, chloroform and anhydrous ether (analytical grade) were ordered from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Polyethylene glycol (PEG, MW5000) was subscribed from Yuanye Biotechnology Co., Ltd. (Shanghai, China).

Instruments

$CsPbBr_3$ QDs were subjected to tip-sonication by ultrasonic cell pulverizer (Scientz-IID, Xinzhi, Ningbo). ^1H NMR spectra were recorded at 400 MHz on a Bruker Avance 400 spectrometer (Bruker, Germany). The PL spectra were collected with a PL spectrophotometer (F-4600, Hitachi, Japan). UV−vis spectra were recorded with Thermo Scientific microplate reader (Thermo Fisher, Xiamen). Transmission electron microscopy (TEM) images were recorded on an HT7700 Exalens TEM (Hitachi, Japan). X-ray diffraction (XRD) was measured with an Ultima IV 285 (Rigaku, Japan). Fourier transform-infrared spectroscopy (FTIR) was conducted on a Thermo Scientific Nicolet iS 50 spectrometers (Thermo Fisher, USA). PLQYs were measured on an Absolute PL quantum yield spectrometer C11347 (HAMAMTSU, Japan). Cell imaging assays was conducted on a Nikon A1 confocal microscopes system (Nikon, Japan) with excitation wavelength at 488 nm. Zebrafish imaging assays was conducted on fluorescent inverted microscope (Nikon ECLIPSE Ti-S, Japan). The colloidal-stability of bio-PQDs were measured with zetasizer (Nano-ZS, Malvern, UK). High-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) image and energy dispersive X-Ray (EDX) mapping were carried out using a Talos F200i microscope (FEI) with a high-brightness field emission gun operated at 200 kV.

Synthesis of $CsPbBr_3$ QDs

The synthesis method was according to previous literature [S1]. Firstly, mineral oil (5 mL) together with OA (0.25 mL) and OAm (0.25 mL) were mixed with Cs_2CO_3 (0.05 mmol) and $PbBr_2$ (0.15 mmol) precursor powders. Then the reaction medium was sonicated (ultrasonic cell pulverizer) at a power of 40 W for 16 min. During the reaction, the colorless reaction mixture

gradually changed to orange-yellow, indicating the successful formation of $CsPbBr_3$ QDs.

Synthesis of PEG-PCL

PEG-PCL was synthesized *via* ring-open polymerization of *ε*-CL using PEG as an initiator according to a previously reported method with minor modifications [S2]. Briefly, *ε*-CL (3 g, 2.5 g, and 2 g respectively for PEG-PCL with different hydrophilic/hydrophobic ratio) and PEG (0.5 g) were added to a polymerization tube, followed by the addition of stannous octoate (0.05 wt% to *ε*-CL) in 50 μL of chloroform. After three cycles of freeze-pump-thaw, the tube was sealed under vacuum and then immersed in an oil bath and stayed thermostatic at 140 ℃ for 20 h. When the polymerization reaction was over, the obtained crude product was dissolved in chloroform followed by precipitating in anhydrous ether, and then was dried thoroughly under vacuum at room temperature (~12 h) to obtain PEG-PCL as a white solid.

Preparation of bio-PQDs

A mixture of PEG-PCL (0.0025 g) and $CsPbBr_3$ QDs (0.1 mmol) in 30 mL of toluene was added to 150 mL of n-hexane, and then the solution was centrifuged at 8000 rpm for 5 min to collect the precipitate, which was dried in vacuum at 37 ℃ for 12 h to obtain pale yellow powder. Subsequently, the powder (5 mg) was dispersed in water (800 μL) and kept under sonication for 4 h to yield the bio-PQDs.

Stability of bio-PQDs

To investigate the PL stability of bio-PQDs in water, in dulbecco's modified eagle medium (DMEM), alkali and acid aqueous solution, bio-PQDs were dispersed in these solutions respectively with a concentration of 6.25 mg/mL and kept at room temperature. At pre-determined intervals, the PL intensities and PLQYs of these solutions were collected.

To investigate the colloidal stability of bio-PQDs in water, bio-PQDs were dispersed in water with a concentration of 6.25 mg/mL and kept at room temperature, and then their size distributions were measured using a zetasizer at pre-determined intervals.

Cell viability assays

Human lung cancer cells (HFF-1) were cultured in DMEM with 10% of heat-inactivated fetal bovine serum (FBS) and 100 μg/mL of penicillin/streptomycin at 37 ℃ under a 5% CO_2 and 95% relative humidity atmosphere. The cells were seeded in a 96-well microplate (200 μL per well, density of 1×10^4 /mL) and cultured for 24 h. Then the medium was replaced by 200 μL of fresh medium containing various concentrations of bio-PQDs. After 24 h of incubation, the medium was removed and 20 μL of MTT solution (5 mg/mL) was added to each well, followed by incubation at 37 °C for 4 h. The supernatant was removed, and 150 μL of DMSO was added to each well to dissolve the formed formazan. After shaking the plates for 10 min, absorbance

values of the wells were measured with a microplate reader at 490 nm. The cell viability was calculated by comparing the absorbance value of the bio-PQDs treated group to that of the control group.

H_2S-responsive behavior

H_2S standard solutions with different concentrations were initially prepared using Na_2S as the source. Then 100 μL of Na_2S solution with a concentration ranging from 0 to 32 μM was added to the as-prepared bio-PQDs solution (6.25 mg/mL, 50 μL), and the obtained mixture was reacted at room temperature for 1 min and the PL emission spectra were collected. To analyze the product of the reaction between H_2S and the bio-PQDs, the bio-PQDs solution treated with 32 μM Na_2S was collected and then analyzed by HAADF-STEM and EDX mapping.

In vitro H_2S-responsive PL imaging

HFF-1 cells were seeded onto cell culture dishes with a density of 8×10^4 cells per dish and incubated for 24 h before treatment. Then the culture medium was discarded and filled with fresh medium containing bio-PQDs (100 μg/ml). After incubation for different time periods, the medium was removed, and the cells were washed with PBS for three times to remove excess bio-PQDs. Afterwards, one group of the cells were incubated with a fresh medium containing Na_2S (30 μM) for 1 h, and the other group was without any treatment. Subsequently, the cells were fixed with paraformaldehyde and treated with DAPI to label the cell nucleus. Finally, the cells were washed three times with PBS and directly observed by Confocal Laser Scanning Microcopy (CLSM).

In vivo H_2S-responsive PL imaging

In vivo H_2S-responsive PL imaging of the bio-PQDs were carried out using zebrafish larvae (3 to 5 days) as a model. Zebrafishes were kindly provided by Institute of Life Science, Fuzhou University. All experiments involved zebrafish were performed in compliance with guidelines from national standard《Laboratory Animal-Requirements of Environment and Housing Facilities》(GB 14925-2001) and Fujian Administration Rule of Laboratory Animal. All the experiments were approved by Experimental Animal Ethics Committee of Fuzhou University. Zebrafishes were cultured in E3 medium at 28 °C. To study the uptake of nanoprobe by zebrafish, zebrafish larvae was placed into a 96-well culture plate, and 200 μL of medium containing bio-PQDs (0.05 mg/mL) was added to each well, followed by incubation at 28 °C for different time periods. Then the medium was removed, and the zebrafish larvae was washed with 200 μL of E3 medium for 3 times. Subsequently, the zebrafishes were directly observed by fluorescent inverted microscope. Similarly, the PL imaging of the bio-PQDs in response to H_2S was studied by adding one step of treating the zebrafishes with a fresh medium containing

Na_2S (15 μM and 30 μM) for 30 min after the treatment of bio-PQDs.

Optimization of the reaction conditions

Relative PL intensity indicates the ratio value of the PL after 1 h to the initial PL and it is selected as an indicator of the stability of the perovskite complex in water. That is, the closer this value to 1 is, the better the water stability of the complex is. To obtain the best stability of the bio-PQDs in water, the effects from the experimental conditions, such as the ratio of PEG-PCL to $CsPbBr_3$ QDs, temperature of water and the sonication time of bio-PQDs in water were optimized. Firstly, the effect of the ratio of PEG-PCL to $CsPbBr_3$ QDs on the stability of the nanocomposite in water was optimized. Figure S1a shows that the complex had the highest water stability when the ratio of PEG-PCL to $CsPbBr_3$ was 0.0025 g/mL, hence this number is chosen for this study. Besides, water temperature is also a important factor influencing the stability of the complex in water. Figure S1b shows that the value of relative PL intensity increased gradually at first and then reached a platform at 25 ℃. Therefore, 25 ℃ was selected for the subsequent study. In addition, the sonication time of bio-PQDs in water can also affect the water stability of the complex. Figure S1c proves that the water stability of the nanocomposite was best at 4 h after sonication, so the sonication time was set as 4 h in this study.

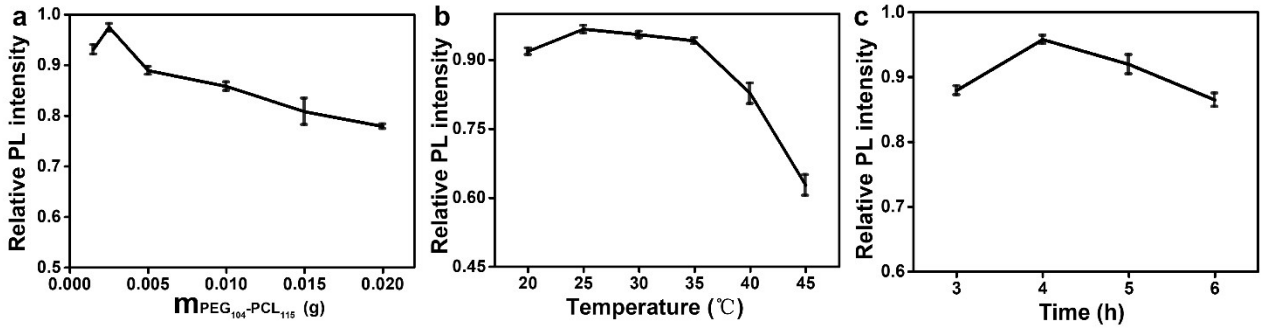

Fig. S1 Changes in PL intensity of bio-PQDs (a) at different concentrations of PEG-PCL; (b) at different temperatures of water; (c) at different ultrasound time. Error bars represent the standard error derived from three repeated measurements.

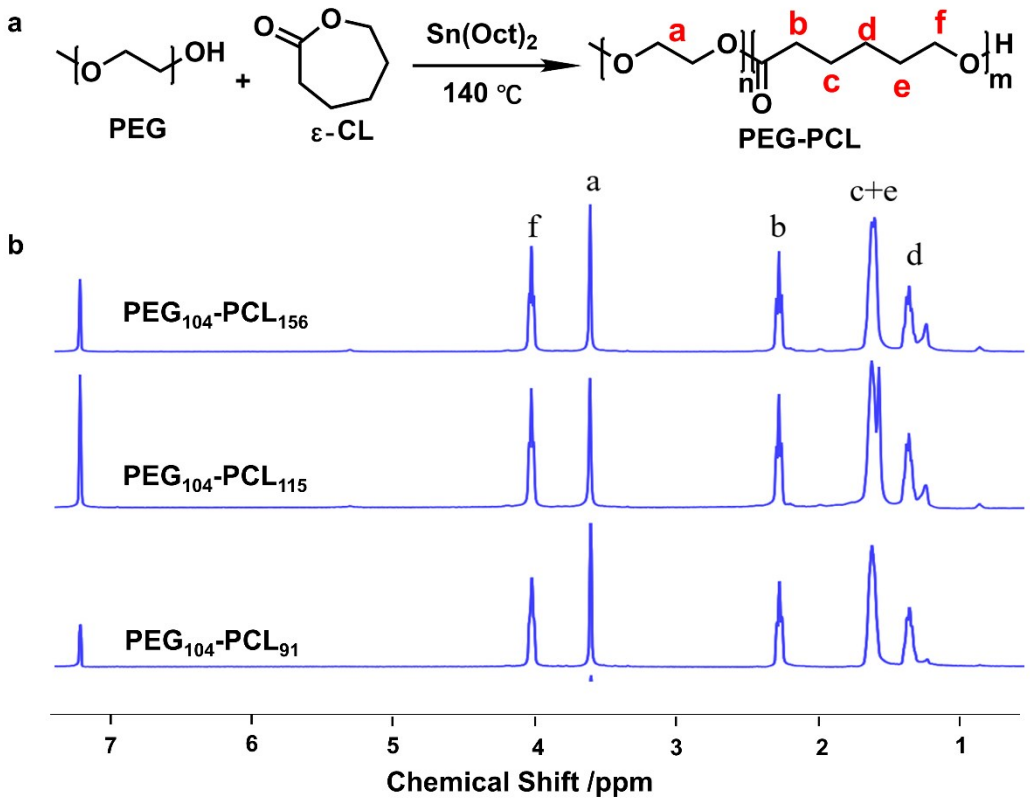

Fig. S2 (a)The synthetic route of PEG- PCL; (b)^{1}H NMR spectra of PEG-PCL in $CDCl_3$.

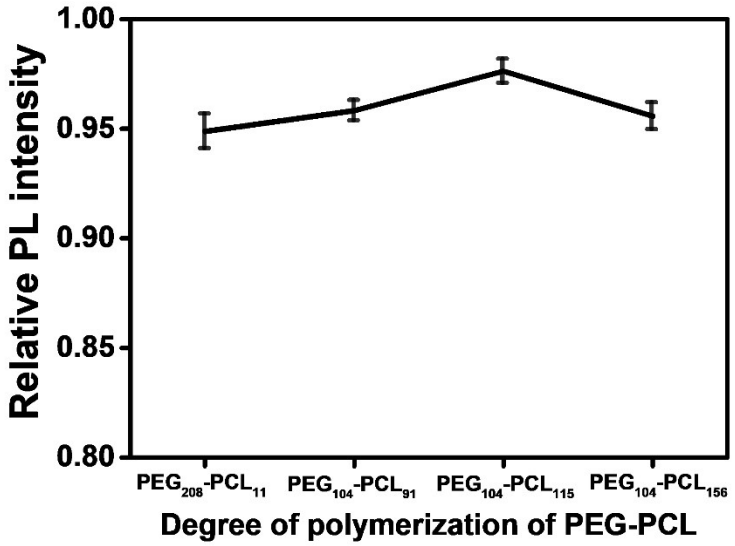

Fig. S3 Changes in photoluminescence intensity at different degree of polymerization of PEG-PCL of bio-PQDs in water within an hour. Error bars represent the standard error derived from three repeated measurements.

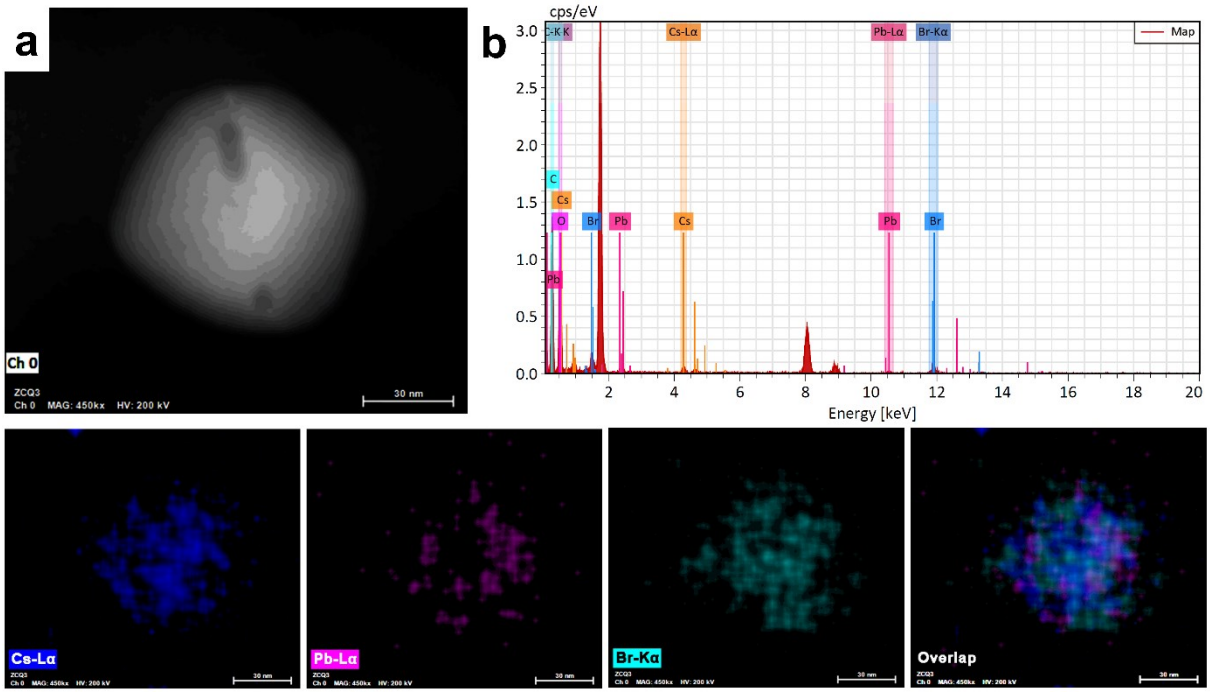

Fig. S4 (a) HAADF-STEM image and (b) EDX mapping of the bio-PQDs.

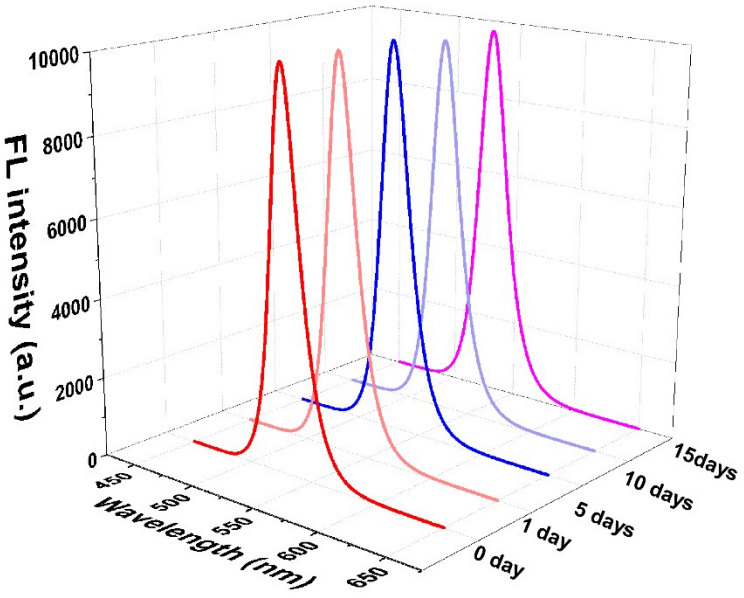

Fig. S5 PL peak intensity for bio-PQDs in water from 0 to 15 days.

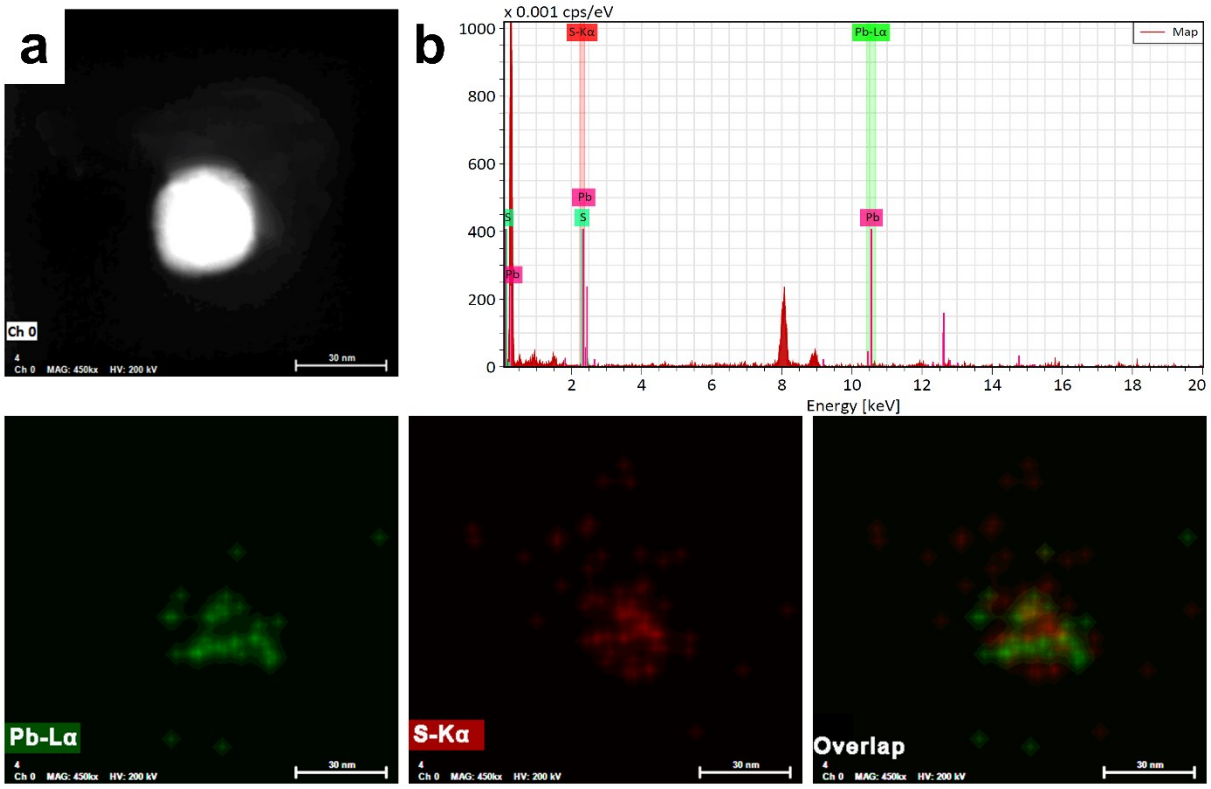

Fig. S6 (a) HAADF-STEM image and (b) EDX mapping of the PBS formed by reaction of H_2S and bio-PQDs.

Table S1 Comparison of photoluminescence properties between PQDs and bio-PQDs.

Sample	PL Lifetime [ns]	FWHM [nm]	PLQY [%]
PQDs	59.35	18.26	42.7
bio-PQDs	87.89	17.48	54.1

Table S2 Table of photoluminescence properties of PQDs reported in the literature.

Composites	**size [nm]**	**Stability (residual PL intensity)**	**PLQY [%]**	**FWHM [nm]**	**PL Lifetime [ns]**	**Ref.**
$CsPbBr_3$@PEO-PPO-PEO	55	84.3% (192 hours)	53	23	/	[S3]
$CsPbBr_3$@NH_4Br	8 ~ 40	40% (3.5 hours)	64.21	23	47.85	[S4]
$CsPbBr_3$-TDPA	3.5 ~ 5.0	75% (5 hours)	58	23	/	[S5]
$CsPbBr_3$-SLN	700 ± 240	80% (10 hours)	13	/	/	[S6]
$CsPbBr_3$/Cs_4PbBr_6	20 ~ 100	80%（7 days）	40	16	/	[S7]
bio-PQDs	50	99% (15 days)	54.1	17.48	87.89	Our work

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