Supporting Information:
Near-infrared-IIb probe affords ultrahigh contrast inflammation imaging
Cong Hua,*1 Biao Huang,2 Yingying Jiang,3 Shoujun Zhu,3,4 Ran Cui*2

1Department of Surgical Neuro-oncology, The First Hospital of Jilin University, Changchun, 130061, PR China
E-mail: huacong@jlu.edu.cn
2College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430070, P.R. China
Email: cuiran@whu.edu.cn
3State Key Laboratory of Supramolecular Structure and Materials, College of Chemistry, Jilin University, Changchun 130012, China
4Key Laboratory of Organ Regeneration & Transplantation of the Ministry of Education, The First Hospital of Jilin University, Changchun, 130061, PR China

The current manuscript mainly focuses on NIR-IIb imaging of vessel and inflammation. The synthesis protocol has been previously reported.1, 2

Materials
PbCl2, CdO, and sulfur powder were purchased from Alfa. Oleylamine, oleic acid, 1-octadecene (ODE), tetrachloroethylene (TCE), poly(acrylic acid) (MW = ~1,800), N,N’-dicyclohexylcarbodiimide (DCC), N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride (EDC), 2-(N-morpholino)ethanesulfonic acid (MES) hydrate were purchased from Sigma-Aldrich. Indocyanine green (ICG, modified) for human injection was purchased from Dandong Yichuang Pharmaceutical Co., Ltd. Phosphate buffered saline (PBS) was purchased from HyClone. Bovine serum albumin was purchased from Sigma-Aldrich. Fetal bovine serum was purchased from Neuromics. The 3.5 kDa dialysis bag (Fisherbrand™ Regenerated Cellulose Dialysis Tubing (21-152-10)) was purchased from ThermoFisher.

Synthesis of quantum dots2
PbS QDs were synthesized by using a procedure slightly modified from that previously described.1, 2 Sulfur precursor solution was prepared by mixing 0.08 g (5 mmol) of sulfur powder and 7.5 mL of oleylamine in a two-neck flask at 120 °C under argon for 30 min. The lead
precursor solution was prepared by mixing 0.834 g (3 mmol) of PbCl$_2$ and 7.5 mL of oleylamine in a three-neck flask and degassing for 30 min under argon at 120 °C and then increasing the temperature to 160 °C. Thereafter, 2.25 mL of the sulfur precursor solution (0.75 mmol of S) was injected into the Pb precursor solution (3 mmol of Pb) under stirring. The temperature was maintained at 160 °C throughout the reaction. After 30 min, the reaction was stopped by the addition of 10 mL of cold hexane and 20 mL of ethanol. The products were collected by centrifugation and re-suspended in a mixture of 10 mL hexane and 20 mL oleyic acid. The mixture was agitated for 10 min to remove excess sulfur from the products. The QDs were precipitated by centrifugation. The precipitation procedure with oleyic acid was repeated three times until the supernatant was colorless. After centrifugation of the suspension and decantation of the supernatant, the QDs were re-suspended in ODE.

PbS/CdS quantum dots (CSQDs) were synthesized via the cation-exchange procedure.$^2$ CdO (1.2 g, 9.2 mmol), oleyl acid (8 mL), and ODE (20 mL) were heated to 200 °C, purged with argon, and then cooled down to 100 °C. Five milliliters of previously prepared PbS QDs suspended in ODE was bubbled with argon for 10 min, and then added to Cd precursor. The reaction flask was quenched with 5 mL of cold hexane after the reaction was conducted at 100 °C for 30 min. PbS/CdS CSQDs were precipitated with ethanol and then resuspended in hexane.

OPA was synthesized with a modified procedure.$^2$ Typically, 0.9 g of poly(acrylic acid) powder (average MW = ~1,800) and 1.56 g DCC was transferred into a round-bottom flask. Ten milliliters of DMF was added to dissolve the mixture. About 1.2 mL of oleylamine was added dropwise into the reaction flask. The molar ratio of oleylamine to PAA was 30%. The solution was stirred overnight for 16 h. Fifty milliliters of 0.5 M HCl was added to the reaction solution. The precipitate was separated by centrifugation, re-dissolved in 3 mL of methanol, and then 20 mL of 1 M HCl was added to the solution. The precipitate was separated by centrifugation. This procedure was repeated at least five times. The final precipitate was dissolved in 5 mL of chloroform and washed by 10 mL of 1 M HCl. The organic phase was collected and dried over anhydrous Na$_2$SO$_4$. Chloroform was removed under vacuum, and the white solid was collected. The average MW of OPA was ~3,000 as determined by gel permeation chromatography. For surface modification, the as-synthesized CSQDs (5.0 mg) were dissolved in 2.0 mL of chloroform
containing 15 mg of OPA. The mixture was stirred at room temperature for 30 min, and the solvent was removed under vacuum by a rotary evaporator. The residue was then dissolved in 2 mL of a 50 mM sodium carbonate solution under sonication. The CSQDs were precipitated by centrifugation at 50,000 rpm for 1 h, and washed three times with deionized water. The purified product was dissolved in 0.01 M MES buffer (pH 8.5) and stored at 4 °C.

**PEGylation of CSQDs**

OPA-modified CSQDs (5 mg) were dissolved in 20 mL of 0.01 M MES buffer (pH 8.5). Fifteen milligram of mPEG-amine (MW = ~5,000) and 5 mg of 8-Arm PEG-amine (MW = ~40,000) with a molar ratio of 24:1 were dissolved in 1 mL of MES, and gradually added to the solution of QDs with stirring. Ten milligram of EDC was dissolved in 500 μL of MES and gradually added to QD solution with stirring. The mixture was stirred at room temperature overnight. The PEGylated QDs were purified by using a 100 kDa filter and washed five times with 1× PBS to remove excess reactants. The purified product was dissolved in 1× PBS and stored at 4 °C.

**NIR-II imaging**

Mice were anesthetized with isoflurane (or chloral hydrate) and placed on a stage with a catheter for intravenous injection of imaging agents. For each imaging experiment, at least three mice were used per group. All NIR-II images were collected on a two-dimensional InGaAs array (Princeton Instruments) based on a previous set-up. Laser excitation proceeded at a wavelength of 808 nm laser and power density (before filters) of ~0.15 W/cm². Emission was typically collected with 1,500 nm long pass filters. A lens set was used for obtaining tunable magnifications ranging from 1× (whole body) to 2.5× (high magnification) by changing the relative positions of two NIR achromats (200 mm and 75 mm, Thorlabs). A variable exposure time was used for the InGaAs camera to capture images in the NIR-IIb window. The acquired images were further processed with Light Field Acquisition software. Pilot study in terms of whole body imaging was performed based on our previous study in NIH. The *in vivo* experiment was approved by the Animal Experiment Regulations of Jilin University. The
inflammation model was established based on previous reports.\textsuperscript{5, 6} Tumors were formed by inoculating $2 \times 10^6$ tumor cells subcutaneously into nude mice.
**Figure S1.** Multilayer surface modification improves *in vivo* stability of QDs. a) OPA coated QDs possess short vessel circulation time. b) Further PEGylation greatly enhances the circulation time and *in vivo* stability of the NIR-IIb QD probe (Same mouse in Fig. 2b).
Figure S2. Whole-body rotating images after administration of the NIR-IIb QD probe into the tail-vein. The improved stability and longer circulation time of the probe allow vessel visualization with super-high imaging quality. In the revised version, we conducted a new set of imaging with the new cohort of mice (n=5). Please also check Video S2 and Video S3.
Figure S3. Selected time points from video-rate NIR-IIb imaging (1,500nm long-pass filter) of a mouse (Fig. 1d, Video S1) after an intravenous injection of NIR-IIb QD probe.
Figure S4. The low amount of PEGylation precluded high-quality NIR-IIb imaging, particularly during later post-injection time points, and the skin signal appeared at 4 to 20 h post-injection time points.
Figure S5. Imaging of a SKOV3 tumor-bearing mouse by PEGylated NIR-IIb QDs. The longer circulation facilitated high accumulation of the probe to visualize the subcutaneous tumor. a) Whole-body NIR-IIb imaging of a SKOV3 tumor-bearing mouse at 1× magnification at different post-injection time points. b) Whole-body NIR-IIb imaging of a SKOV3 tumor-bearing mouse at an early time point after QD probe injection. c) Tumor signal time course at different post-injection time points. Data are presented as the mean ± standard error of the mean.
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