Supplementary Information

Stable DHLA-PEG capped PbS quantum dots: From synthesis to near-infrared biomedical imaging

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List of abbreviation: LA, lipoic acid; DHLA,dihydrolipoic acid; PEG, polyethylene glycol; DCM, dichloromethane; TLC, thin layer chromatography; NMR, nuclear magnetic resonance.

Experimental section

Experimental techniques: All reagents were obtained from Sigma Aldrich and were used without further purification. TLC analysis of reaction mixtures was performed using Merck Kieselgel 60 F254 plates; visualization was carried out by irradiation with a UV lamp (254 nm) and staining with phosphomolybdic acid or permanganate solution. NMR were acquired using a 400MHz Avance or a 600 MHz Avance III spectrometer equipped with broadband observe probes. ^{1}H acquisition was achieved using a 30 degree tip angle pulse with recycling delays of 5 s. All spectra were processed and analyzed with a combination of Topspin and MNova software. Curve fitting was achieved using Prism software. For the transmission electron microscopy (TEM), the nanoparticles were deposited on a graphene-oxide coated grid and images were recorded on a JEOL 2100F FEGTEM (200 kV). Optical properties of quantum dots were measured in solution with optical excitation provided by a He-Ne laser at 633 nm and power $P \approx 10$ Wm^{-2}. The luminescence was dispersed by a 150 g/mm grating and detected by either a nitrogen-cooled (InGa)As array photodiode or a charge-coupled device (CCD).

Aqueous solutions of PbS were stored at T = 4 °C under nitrogen atmosphere. Quantum yield measurements were performed using Edinburg Instrument fluorescence spectrometer system FLS980 equipped with 120 mm diameter integrating sphere.

Synthesis of PbS QDs with DHLA-PEG400-R: DHLA-PEG400-R (0.129 mmol), where R is -OMe, -NH_2, -COOH, was dissolved in water (10 ml) and the solution stirred and degassed with nitrogen for 15 minutes. Pb^{2+} (0.43 mL, 0.1M of $Pb(OAc)_2$ in water) was added and the pH was adjusted with trimethylamine (TEA) to pH = 11. Aqueous solution of Na2S was added dropwise to form the nanocrystals: in order to form the QDs of different sizes, the ratio of Pb^{2+} to S^{2-} was varied from 1:0.3 (0.26 mL, 0.05 M Na_2S) to 1:0.6 (0.52 mL, 0.05 M Na_2S).

Near-infrared imaging: A home-built second-NIR fluorescence microscope used is based on the Macro Zoom System at magnification of 0.63x (MVX; Olympus) and is equipped with an InGaAs CMOS camera (C10633-34; Hamamatsu photonics KK). A customized filter set consisting of an excitation filter for the 670 nm laser, dichroic mirror to reflect the 670 nm laser, and a band-pass emission filters (1000 ± 25 nm and 1300 ± 25 nm) was used for imaging. 670 nm laser diodes (BWF1 series; B&W TEK) were used to provide excitation light. Maximum excitation powers on the sample stage were 25.5 mW/cm^2. Device control and data acquisition were performed by HCImage (Hamamatsu photonics) software and a customized LabVIEW program (National Instruments). Images were processed using Fiji software. [1]

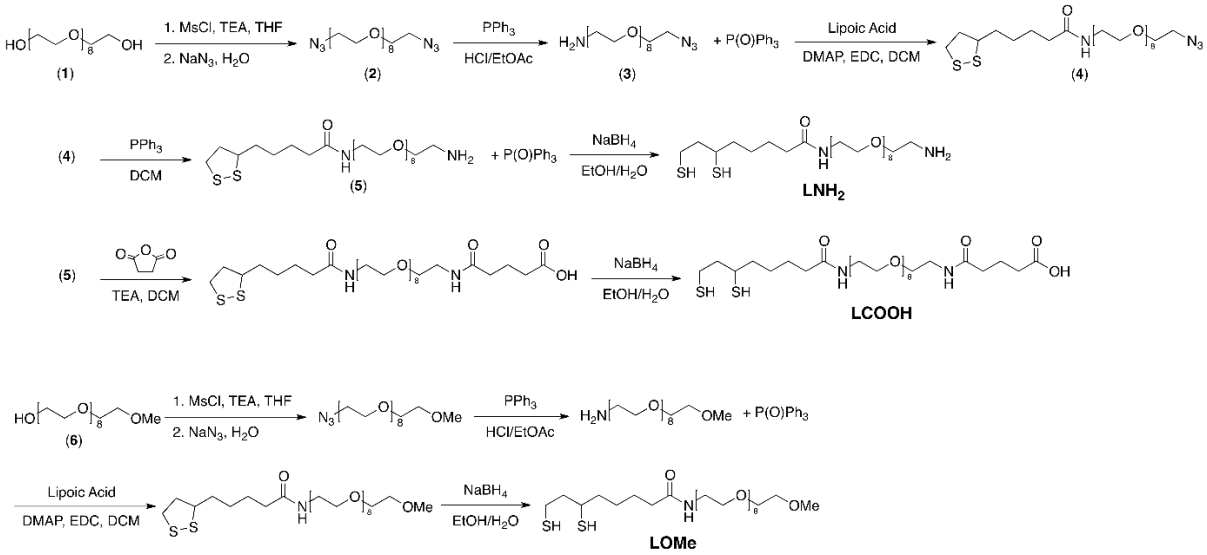
2-month old female BALB/c nu/nu mice (Charles River Japan) were injected with 30 μL of a 0.2 mM QD solution *via* a tail vein. For *in vivo* imaging, special bait without alfalfa (Oriental yeast, Tokyo, Japan) were fed to all of mice until the onset of experiment. Experiments were conducted after one week of its feeding. Second-NIR fluorescence images (ex: 670 nm, emission > 1000 nm) of anesthetized mouse were taken immediately after injection, and 24 h and 48 h after the injection. Mice were then humanely killed and organs removed for *ex vivo* imaging. Liver and brain tissue from untreated mice were cut into slices of defined thicknesses

between 0.1 mm and 4 mm using a microslicer (DTK-1000; Dosaka EM). Slices of different thickness were then overlaid in turn over sealed capillaries containing QD solutions at 1 mg/ml. All experiments were performed in compliance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and were approved by RIKEN Animal Care and Use Committee.

[1] Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J.-Y.; White, D. J.; Hartenstein, V.; Eliceiri, K.; Tomancak, P.; Cardona, A., Nat. Methods **2012**, 9 (7), 676–682.

SI1 Characterisation of the DHLA-PEG-based ligands

Syntheses were performed following the procedure from References [2] and [3] (Scheme 1), and the products were characterized using NMR and TLC. ^{1}H NMR was performed at 400 MHz in $CDCl_3$. TLCs were carried out using a 10:1 (v/v) DCM/methanol eluent.



Scheme S1 Scheme of the reactions used for ligand synthesis.

1) N_3-PEG400-N_3 was synthesised in a yield of 65.5 %. ^{1}H NMR: δ 3.62-3.75 (m), 3.41 (t, 4H, *J* = 5.0 Hz). N_3-PEG400-N_3 was used to produce N_3-PEG400-NH_2. TLC Rf~ 0.1. ^{1}H NMR: δ 3.61-3.89 (m), 3.53 (t, 2H, *J* = 5.0 Hz), 3.40 (t, 2H, *J* = 5.0 Hz), 2.88 (t, 2H, *J* = 5.2 Hz).

2) LA-PEG400-N_3 was synthesised in 71% yield. TLC Rf~ 0.6. ^{1}H NMR: δ 6.29 (br s, 1H) 3.62-3.8 (m), 3.55-3.60 (m, 4H), 3.47 (t, 2H, *J* = 5.2 Hz), 3.41 (t, 2H, *J* = 5.4 Hz), 3.09-3.23 (m, 2H), 2.49 (m, 1H), 2.21 (t, 2H, *J* = 7.4 Hz), 1.93 (m, 1H), 1.71 (m, 4H), 1.49 (m, 2H).

3) LA-PEG400-NH_2 was obtained in 73% yield. TLC Rf~ 0.1. ^{1}H NMR: 3.5-3.73 (m), 3.44-3.5 (m, 4H), 3.09-3.24 (m, 2H), 2.87-3.0 (m, 2H), 2.63-2.76 (m, 2H), 2.42-2.54 (m, 1H), 2.18-2.30 (m, 2H), 1.88-1.98 (m, 1H), 1.60-1.79 (m, 4H), 1.40-1.56 (m, 2H).

4) LA-PEG400-COOH was synthesised in 93% yield. TLC Rf~ 0.25. ^{1}H NMR: 6.90–7.30 (br s, 1H), 6.20–6.70 (m, 1H), 3.60–3.74 (m), 3.52–3.60 (m, 5H), 3.44 (m, 4H), 3.07–3.22 (m, 2H), 2.66 (m, 2H), 2.55 (m, 2H), 2.41–2.51 (m, 1H), 2.21 (m, 2H), 1.83–1.96 (m, 1H), 1.57–1.76 (m, 4H), 1.38–1.53 (m, 2H).

5) N_3-PEG500-OMe was synthesised in quantitative yield. ^{1}H NMR: δ 3.69-3.65 (m, 32H), 3.57-3.55 (m, 2H), 3.41 (d, J = 5.3 Hz, 1H), 3.40 (s, 3H). N_3-PEG500-OMe was used to produce NH_2-PEG500-OMe (41% yield). ^{1}H-NMR: δ 3.71-3.62 (m, 50H), 3.57-3.55 (m, 3H), 3.53 (t, J = 5.2 Hz, 2H), 3.39 (s, 4H), 2.88 (t, J = 5.2 Hz, 2H).

6) LA-PEG500-OMe was synthesised in quantitative yield. ^{1}H NMR: δ 3.76-3.60 (m, 40H), 3.59-3.55 (m, 4H), 3.49-3.44 (m, 2H), 3.40 (s, 3H), 3.23-3.10 (m, 2H), 2.52-2.44 (m, 1H), 2.39-2.32 (m, 1H), 2.23-2.19 (m, 2H), 1.97-1.88 (m, 1H), 1.75-1.63 (m, 4H), 1.54-1.43 (m, 2H).

All ligand molecules were synthesized under N_2 atmosphere. The LA was reduced to DHLA to provide the anchoring thiol (–SH) groups. Briefly, LA-PEG400-R, where R is –NH_2, COOH or OMe, was dissolved in water/ethanol (5 mL, 4:1 v/v) and sodium borohydride (4 eq.) was added to the solution. After 4 h stirring, brine (10 mL) was added to quench the reaction and the product was extracted with chloroform (5x 20 mL). The analysis of the resulting products is given below.

DHLA-PEG400-OMe (**LOMe**): ^{1}H-NMR: δ 6.29-6.27 (m, 1H), 3.64-3.63 (m, 41H), 3.55-3.52 (m, 4H), 3.43 (q, *J* = 5.2 Hz, 2H), 3.36 (s, 3H), 2.91-2.89 (m, 1H), 2.73-2.62 (m, 2H), 2.24 (d, *J* = 6.4 Hz, 2H), 2.18 (t, *J* = 7.4 Hz, 2H), 1.91-1.86 (m, 1H), 1.77-1.26 (m, 12H).

DHLA-PEG400-NH_2 (**LNH$_2$**): ^{1}H-NMR: δ 3.64 (dd, *J* = 3.9, 1.2 Hz, 24H), 3.56-3.50 (m, 4H), 3.44 (q, *J* = 5.1 Hz, 2H), 2.99 (s, 1H), 2.94-2.89 (m, 1H), 2.86 (t, *J* = 5.2 Hz, 2H), 2.72-2.65 (m, 2H), 2.21-2.17 (m, 2H), 1.92-1.87 (m, 2H), 1.74-1.00 (m, 30H).

DHLA-PEG400-COOH (**LCOOH**): ^{1}H-NMR: δ 3.65-3.61 (m, 14H), 3.54 (t, *J* = 4.9 Hz, 2H), 3.42 (d, *J* = 4.8 Hz, 2H), 2.93-2.87 (m,), 2.70 (ddt, *J* = 12.6, 8.6, 4.6 Hz, 1H), 2.66-2.63 (m, 1H), 2.53-2.51 (m, 1H), 2.20-2.18 (m, 1H), 1.92-1.84 (m, 1H), 1.76-1.40 (m, 5H), 1.34 (t, *J* = 8.0 Hz, 1H), 1.31 (s, 1H), 1.24 (s, 3H).

[2] Susumu, K.; Mei, B. C.; Mattoussi, H. *Nat. Protoc.* **2009**, *4*, 424-436.

[3] Mei, B. C; Susumu, K.; Medintz, I. L.; Mattoussi, H. *Nat. Protoc.* **2009**, *4*, 412-423.

SI2 Ligand stability study by NMR

The stability of all DHLA-PEG capping ligands was probed by time-dependent ^{1}H NMR studies. Sample consisted of ligand molecule (0.1 g) dissolved in $CDCl_3$ (0.6mL). NMR were recorded using a Bruker 400 MHz or 600 MHz spectrometer. Data were obtained at 25 °C and averaged over 16 transients using a 30 degree tip angle pulse with recycling delays of 5s, with spectra being recorded every 30 min. For all NMR experiments, referencing was done by comparison to the 2H-lock nucleus frequency. The spontaneous oxidation reaction is shown in Figure S1. The changes observed over 48 h of the ligand in solution by NMR are shown in Figure S2 and the corresponding kinetic parameters are summarised in the Table S1.

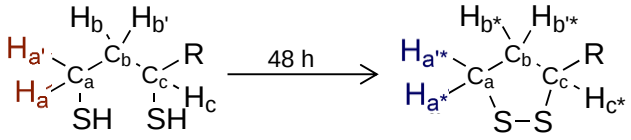


Figure S1 Reaction scheme and proton key for the observed ^{1}H NMR resonance shifts of the dithiolane ring undergoing oxidation in the DHLA part of the ligand molecule.

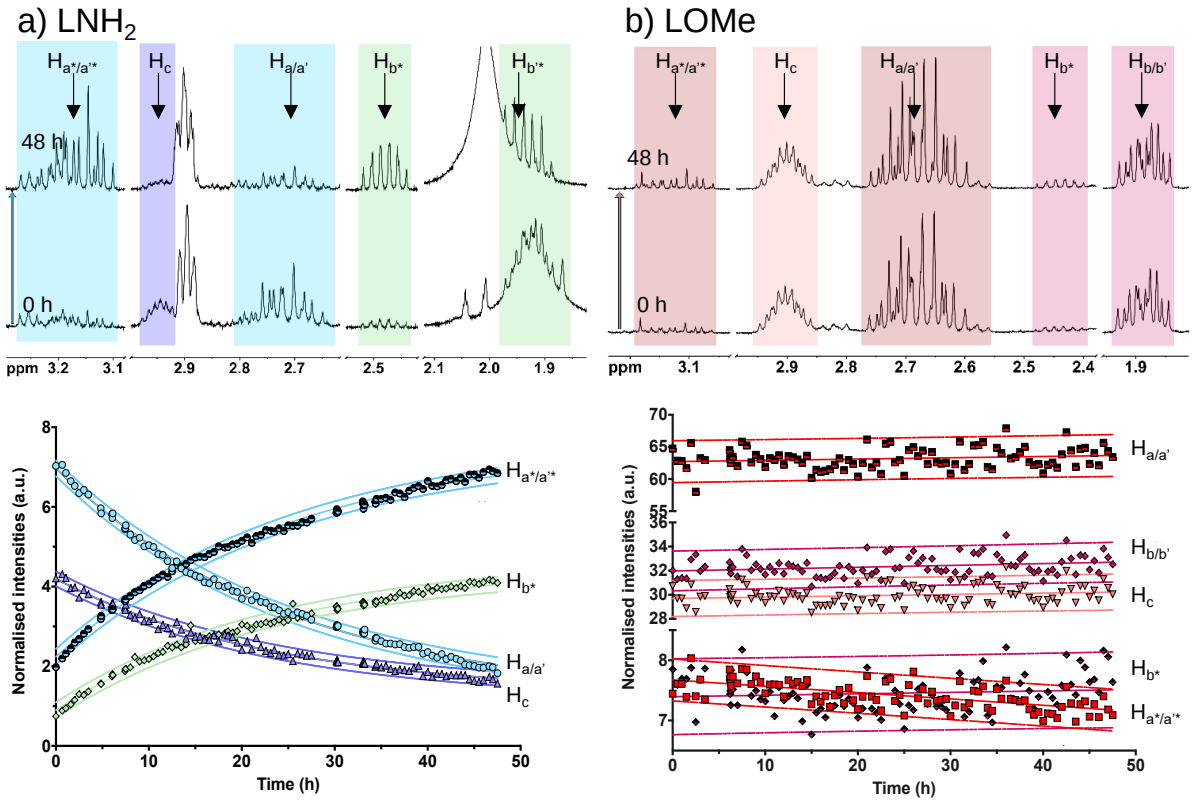


Figure S2 NMR spectra of **(a)** LNH_2 and **(b)** LOMe molecules
and a corresponding time dependence of the intensities of selected resonances.

Table S1 Kinetic parameters describing change of NMR intensity for characteristic resonances for LNH_2

LNH_2	$H_{a/a'}$	H_c	Ha*/a'*	Hb*
k (s^{-1})	140.98 ± 4.38	154.58 ± 8.16	140.29 ± 4.67	152.60 ± 6.90
$t_{1/2}$ (s^{-1})	63720	58104	64044	58860

We attribute the colloidal instability of QDs synthesized using these molecules as capping ligands to oxidation of the DHLA portion of the ligand molecule and to the presence of phosphine and phosphine oxides from the Staudinger reduction of the azide groups. The presence of phosphine oxides in the ligand solution is confirmed by ^{31}P NMR (Figure S3). Binding of phosphine oxides to the nanocrystals surface makes it hydrophobic, thus leading to their precipitation from the aqueous solution.

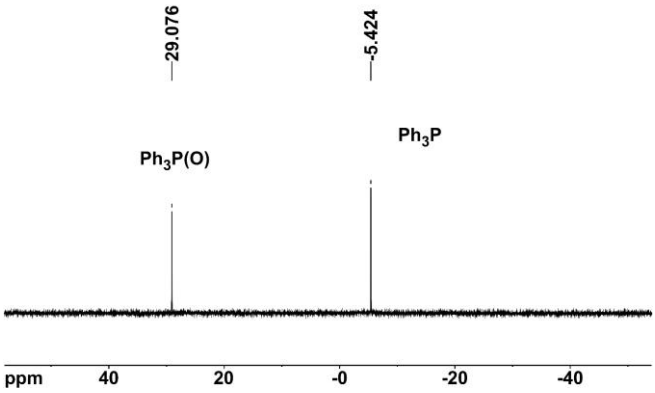


Figure S3 Decoupled ^{31}P NMR spectra of LNH_2, showing characteristic resonances for triphenyl phosphine and triphenyl phosphine oxide at -5.42 ppm and -29.07 ppm, respectively.

SI3 Tissue penetration studies, *in vitro* cytotoxicity and *in vivo* imaging

In order to assess the potential of DHLA-PEG capped PbS QDs for bioimaging applications, we have prepared mouse brain and liver tissue slices with thickness varying from 0.2 mm to 4 mm. The slices were placed over ~1 mm diameter glass capillaries containing LNH_2 QD solutions with photoluminescence centered at 1050 nm and at 1200 nm and they were then imaged (Figure S4).

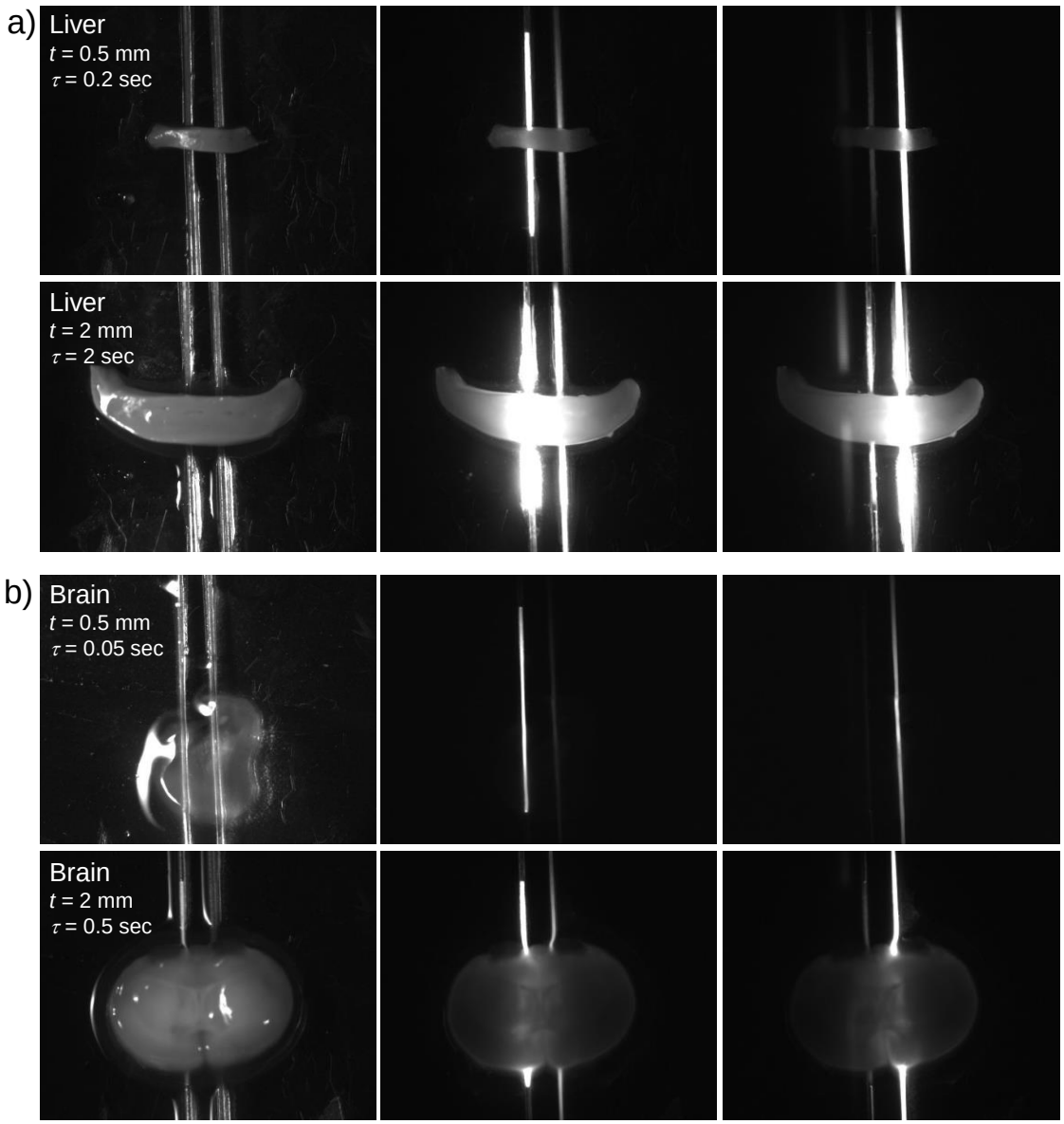


Figure S4 Bright field images (left images) and corresponding near-infrared (NIR) images detected at 1000 ± 25 nm (middle images) and at 1300 ± 25 nm (right images), through brain (a) and liver (b) tissue slices of different thickness.

Figure S5 shows the MTT profile for three studies cell lines exposed to PbS-LNH_2 QDs over a period of 72h.

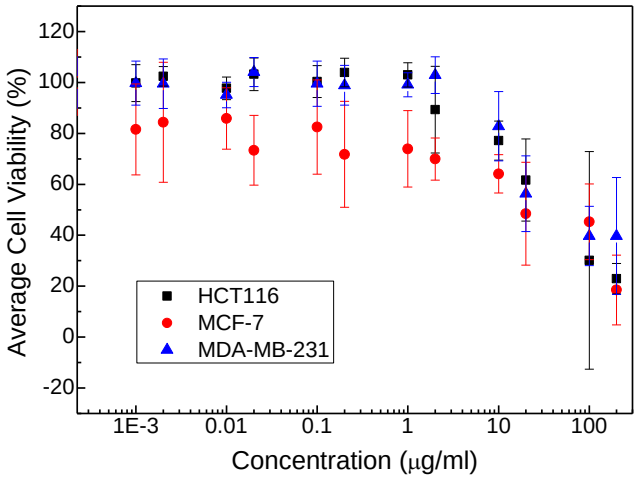


Figure S5 *In vitro* assessment of cytotoxicity of PbS-LNH_2 QDs using MTT assay.

Immediately following injection of QD solutions via tail vein, the NIR imaging of mice revealed emission in was observed in internal veins ~ 0.5 - 1 cm below the skin/skull as well as facial veins/capillaries closer to the surface (Figure S6). No measureable emission was observed in brain after ~ 30 sec after injection.

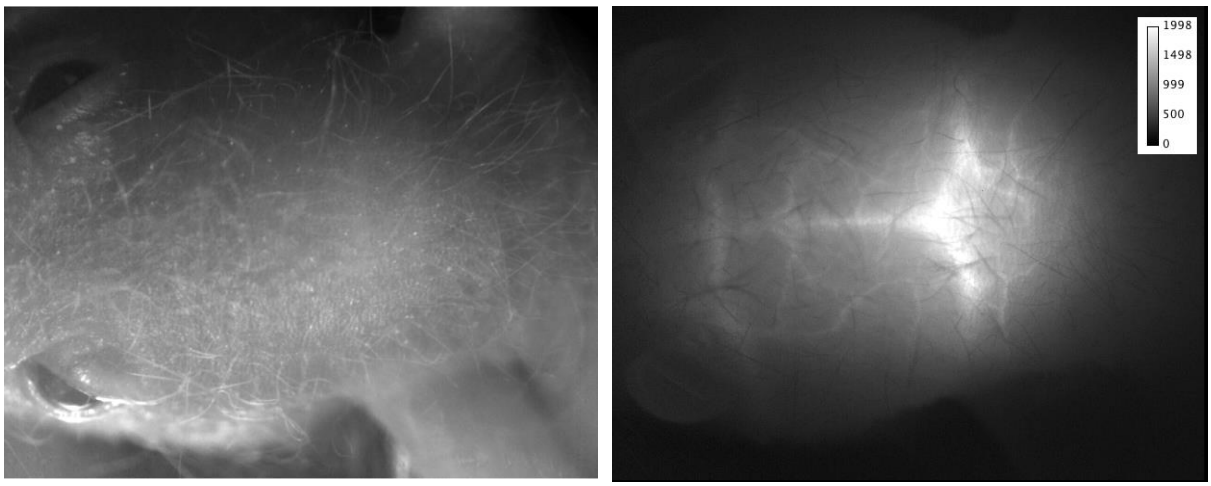


Figure S6 A photograph of mice head and corresponding NIR image recorded at l > 1000 nm immediately after injection of QD solution.

Figure S7 shows typical photoluminescence images of control mouse and that injected with the QD solution. The photoluminescence was detected only in QD-injected mice, and was observed in the areas of liver and spleen and in corresponding organs *ex-vivo*. In some QD-injected mice we also observed NIR light emission in the bladder area (Figure S8), suggesting that QDs are cleared from the body *via* the urinary tract.

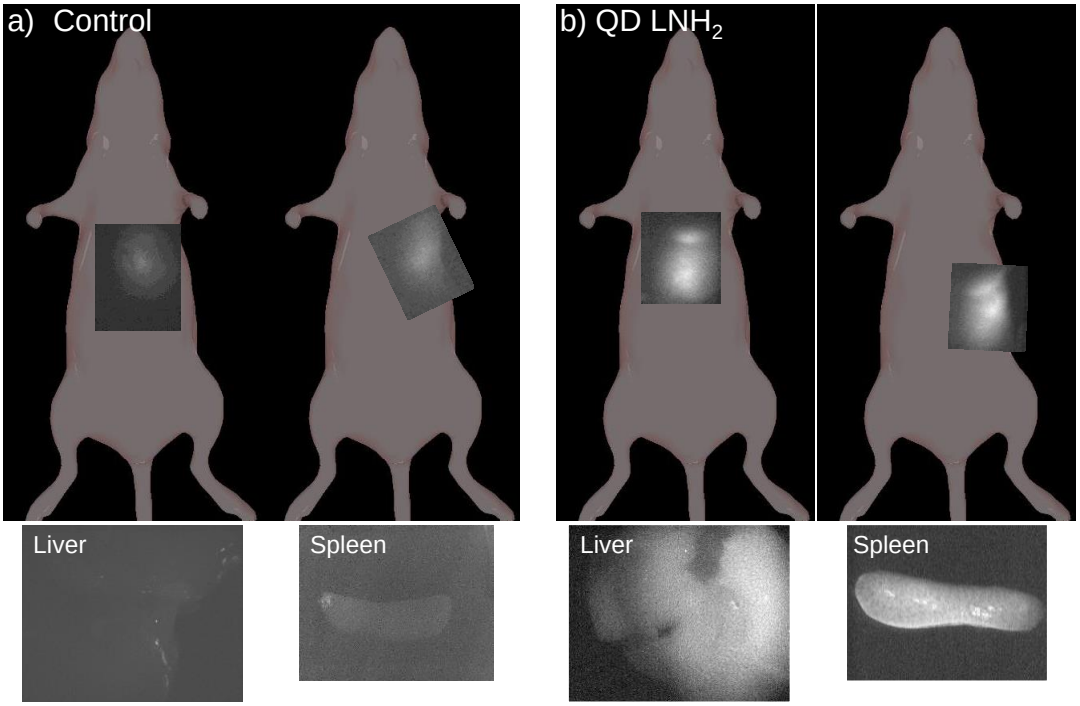


Figure S7 *In vivo* near-infrared images of **(a)** control mouse and **(b)** mouse injected with LNH_2 QDs (PL emission at 1250 nm) following 24 h exposure. Insets show corresponding *ex-vivo* NIR images of spleen and liver.

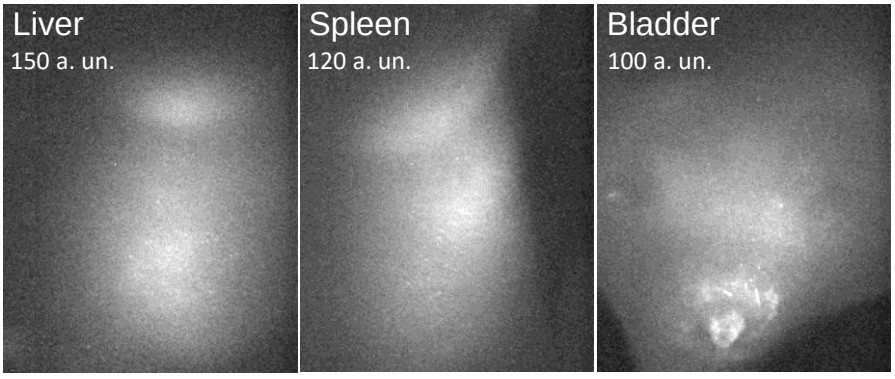


Figure S8 *In vivo* near-infrared images of the areas of liver, spleen and bladder of a mouse 24 h after injection with LNH_2 QDs.