Electronic Supplementary Information

Compact tridentate ligands for enhanced aqueous stability of quantum dots and *in vivo* imaging

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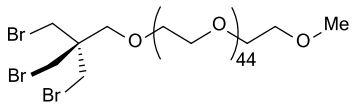
General

Unless otherwise specified, chemicals were purchased from Sigma-Aldrich and used without further purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone before use. Flash chromatography was carried out on Kieselgel 60 (230–240 mesh, Merck). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts (δ) are given in ppm relative to the NMR solvent peak and coupling constants (*J*) in hertz.

Synthesis of tris(mercaptomethyl) polyethylene glycol (TMM–PEG) ligands

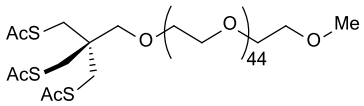
*Synthesis of polyethylene glycol monomethyl ether tribromide **2***



Sodium hydride (60% in mineral oil, 420 mg, 5 equiv.) was washed with pentane and suspended in dry THF (10 mL). A solution of PEG2000 monomethylether (4 g, 2 mmol, 1 equiv.) in dry THF (10 mL) was added to the suspension at room temperature. The mixture was refluxed for 30 min, cooled down to room temperature and stirred for an additional 30 min. The resulting suspension was added to a solution of tetrakis(bromomethyl) methane (7.75 g, 10 equiv.) in dry THF (10 mL) and the mixture was stirred at room temperature for 24 h. The reaction was quenched with water (50 mL), extracted with dichoromethane (3 × 75 mL), dried over magnesium sulfate, filtered, and concentrated under vacuum. Purification by column chromatography (silica gel, gradient CH_2Cl_2/MeOH 99:1→90:10) gave pure **2** as a colorless waxy solid in 87% yield.

^{1}H NMR ($CDCl_3$) δ: 3.70−3.62 (M, 180H; CH_2−O), 3.55 (s, 6H; CH_2−Br), 3.38 (s, 3H; CH_3−O); ^{13}C NMR ($CDCl_3$) δ: 77.5, 71.4−70.0 (multiple C), 59.1, 43.3, 34.2.

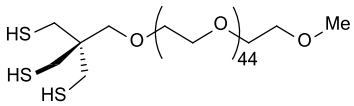
*Synthesis of polyethylene glycol monomethyl ether trithioacetate **3***



Compound **2** (3.48 g, 1.5 mmol, 1 equiv.) and potassium thioacetate (2.57 g, 15 equiv.) were dissolved in dry acetonitrile (40 mL) and stirred overnight at 60 °C. The solvent was removed under vacuum and the residue was taken in dichloromethane (50 mL), washed with water (2 × 20 mL), dried over magnesium sulfate, filtered, and concentrated under vacuum. Column chromatography (silica gel, gradient CH_2Cl_2/MeOH 99:1→90:10) afforded **3** as waxy yellowish solid in 90% yield.

^{1}H NMR ($CDCl_3$) δ: 3.70−3.62 (M, 180H; CH_2−O), 3.38 (s, 3H; CH_3−O), 3.06 (s, 6H; CH_2−S), 2.34 (s, 9H; CH_3−CO); ^{13}C NMR ($CDCl_3$) δ: 193.7, 77.5, 71.5−70.0 (multiple C), 59.1, 42.4, 34.6, 30.6.

*Synthesis of polyethylene glycol monomethyl ether trithiol **1** (TMM-PEG2000)*



Compound **3** (3 g, 1.3 mmol, 1 equiv.) was dissolved in dry THF (30 mL). Lithium aluminum hydride (747 mg, 15 equiv.) was added to the solution and the mixture was stirred at room temperature. After 2 h, the flask was placed in an ice bath and the reaction quenched by slow addition of water (50 mL), followed by 3 **M** hydrochloric acid to reach a pH value of 2–3. The solution was extracted with dichloromethane (5 × 75 mL) and the combined organic layers were dried over magnesium sulfate, filtered, and concentrated under vacuum to give pure **1** as a yellowish waxy solid in 85% yield.

^{1}H NMR ($CDCl_3$) δ: 3.70−3.60 (M, 180H; CH_2−O), 3.40 (s, 3H; CH_3−O), 2.64 (d, *J* = 8.5 Hz, 6H; CH_2−S), 1.33 (t, *J* = 8.5 Hz, 3H; SH); ^{13}C NMR ($CDCl_3$) δ: 77.5, 71.6−70.1 (multiple C), 59.0, 43.3, 27.0.

*Synthesis of polyethylene glycol monomethyl ether dithiol **4** (DHLA-PEG2000)*

DHLA-PEG2000 **4** was prepared according to the procedure described by Mattoussi for analogous compounds.[1]

Synthesis and characterization of quantum dots

CdSe/CdS/ZnS QDs were prepared using previously described syntheses based on the reaction of carboxylate precursors at elevated temperatures and the SILAR protocol.[2,3]

$CuInS_2$/ZnS QDs were synthesized using a variation of a previously reported protocol.[4] Briefly, copper (I) chloride (0.8 mmol), indium chloride (0.8 mmol), tri-*n*-octylphosphine (TOP, 8 mL), oleylamine (OAm, 8 mL), and 1-octadecene (ODE, 40 mL) were mixed in a three-neck flask and degassed for 30 min at 70 °C. Under argon, zinc diethyldithiocarbamate (0.8 mmol) dispersed in TOP (4 mL) was quickly injected at 190 °C and the reaction was quenched after ~ 1 min. The nanocrystals were then washed with ethanol and redispersed in hexane (20 mL). A fraction of this core solution (18 mL) was then mixed with ODE (32 mL) and OAm (4 mL) and degassed. A precursor solution of zinc bis-ethylxanthogenate (2.2 mmol) and zinc chloride (5.9 mmol) in di-*n*-octylamine (4 mL), TOP (12 mL), and ODE (20 mL) was prepared. Under argon, at room temperature, an aliquot of the precursor solution (2 mL) was added to the core solution. The temperature was then raised to 190 °C and the remaining precursor solution was injected dropwise at 10 mL h^{-1}. The produced core/shell nanocrystals were finally washed with ethanol and redispersed in hexane.

QD concentrations were estimated using the absorbance of the core nanocrystals at 350 nm, according to previously published procedures.[5]

Photoluminescence quantum yields were measured using fluorescein in basic ethanol as a standard (97 % quantum yield).[6]

Typical ligand exchange procedure

As prepared QDs (8 nmol in 1 mL hexane) were precipitated in ethanol and resuspended in chloroform (1 mL). An excess of DHLA–PEG or TMM–PEG ligand (0.1 mmol) was added and the solution was heated overnight to reflux (> 61 °C). Ethanol (2 mL) was then added, followed by slow addition of hexane until the solution became turbid. QDs formed a pellet upon centrifugation while excess of ligands remained in the supernatant.[7] QDs were then resuspended in water and purified by ultracentrifugation on 10–40% sucrose gradient for 20 min at 268 000 ×*g* and by ultrafiltration using 30 kDa MW cutoff Vivaspin filters.

Dynamic light scattering (DLS) experiments

Hydrodynamic sizes were measured by DLS using a CGS-3 goniometer system equipped with a HeNe laser illumination at 633 nm (Malvern Instruments, Southborough, MA). The autocorrelation function was performed by an ALV-5000/EPP correlator (ALV, Langen, Germany) and analyzed by the CONTIN algorithm. Results are presented in Figure S1 (see below).

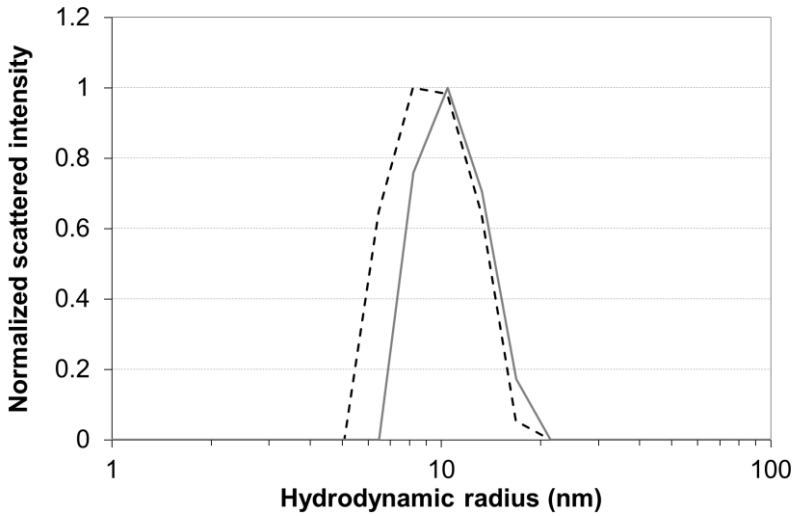


Fig. S1. Size distribution curves obtained by DLS analysis for QDs capped with DHLA–PEG2000 (- - -) or TMM–PEG2000 (—).

Fluorescence stability at different pH values

CdSe/CdS/ZnS QDs capped with DHLA–PEG2000 or TMM–PEG2000 were diluted in Britton–Robinson buffer (H_3BO_3 0.04 M; H_3PO_4 0.04 M; CH_3CO_2H 0.04 M) to reach a QD concentration of 0.25 µM. The pH was then adjusted to different values (1.5, 3, 4, 6, 7, 8, 10, and 12) by addition of 0.2 µM NaOH. After 1 h of incubation, fluorescence spectra were recorded (excitation at 400 nm) for each sample and normalized with the pH 7 sample taken as a 100% reference.

Fluorescence stability with iodine

CdSe/CdS/ZnS QDs capped with DHLA–PEG2000 or TMM–PEG2000 were diluted in deionized water to reach a QD concentration of 0.25 µM before increasing amounts of 1 M iodine in THF were added to reach I_2 concentrations of 33, 50, 67, 83, 100, 133, 167, 233, and 300 µM. After 2 h of incubation, fluorescence spectra were recorded (excitation at 400 nm) for each sample and normalized with regard to an iodine-free sample taken as a 100% reference.

Competition with dithiothreitol (DTT)

CdSe/CdS/ZnS QDs capped with DHLA–PEG2000 or TMM–PEG2000 were diluted in an aqueous solution of 1.5 M DTT and 400 mM NaCl. The aggregation kinetics of both QD types was evaluated by measuring the absorption spectra after 0, 41, 65, 138, 192, 224, 248, 307, 330, and 720 h. All samples underwent mild centrifugation for 5 min at 6 000 ×*g* to eliminate QD aggregates prior to recording the optical density at 400 nm.

Preliminary toxicity test: *In vitro* cell proliferation assay

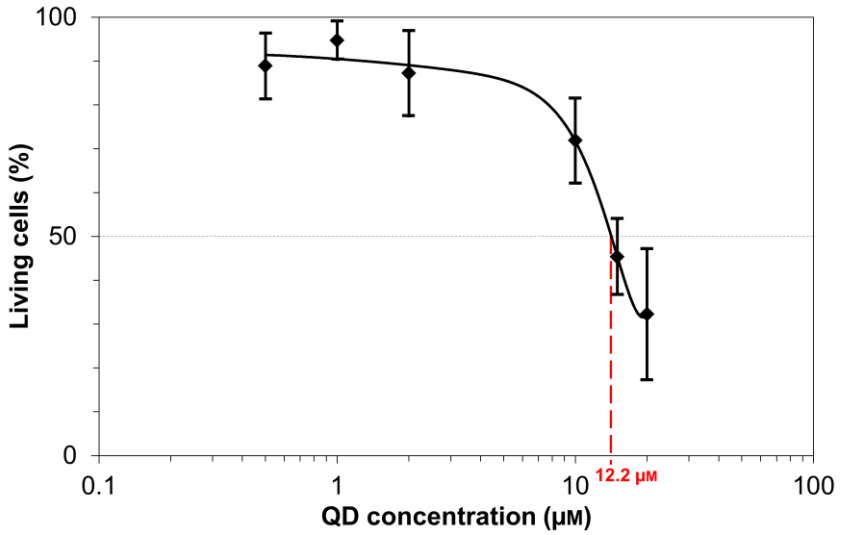


Fig. S2. Preliminary toxicity test results obtained with TMM–PEG2000-capped NIR-QDs. Cell proliferation assay (MTS) on MDA-MB-231 cells; IC_{50} = 12.18 ± 1.06 µM.

In a 96 well plate, 2 × 10^3 cells (MDA-MB-231) diluted in 50 µL of culture medium were deposited per well. After 24 h in a cell incubator, 50 µL of PBS buffer containing TMM–PEG-encapsulated NIR-QDs at different concentrations (20, 10, 2, 1, and 0.5 µm) was added. The plate was then allowed to stand in a cell incubator for 48 h. Then, 20 µL of MTS (tetrazolium compound included in the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay, Promega) was added and the plate was analyzed with a Mithras microplate reader (LB 940, Berthold) at 490 nm after 2 h of incubation in a cell incubator. The data were compared to a well containing only 2 × 10^3 cells in 50 µL of culture medium and 50 µL of PBS buffer and revealed with 20 µL of MTS. All data points were corrected by subtraction

of the absorbance obtained with the corresponding concentration of QDs in 50 µL of culture medium and 50 µL of PBS buffer after 2 h of incubation with 20 µL of MTS (*i.e.* controls without cells). The experiments were repeated 6 times. The plot was expressed as a function of the percentage of living cells, 100% being the well containing only cells and MTS. Results are shown in Figure S2, above.

In vivo **experiments**

All animal use procedures were in strict accordance with the recommendations of the European Community (86/609/CEE) and the French National Committee (décret 87/848) for the care and use of laboratory animals. All mice that were used were female nude mice weighing approximately 23 g and housed under standard conditions with food and water *ad libitum*. Mice were anesthetized with isoflurane (1–3%) and injected intravenously in the tail with TMM–PEG2000-capped $CuInS_2$/ZnS QDs (10 nmol kg^{-1}) in PBS. Under anesthesia, planar fluorescent pictures of mice were taken using the TomoFluo3D optical imager.[8] Planar pictures of fluorescence under excitation at 660 nm and signal recovery using a 700 long pass filter (Schott-RG9) were taken at various time points (2 h, 4 h, and 24 h after injection). Mice were euthanized 24 h after injection and their organs were resected and analyzed using the TomoFluo3D optical imager. The fluorescence intensity was adjusted by subtracting the autofluorescence of the organs measured on a control mouse (non-injected).

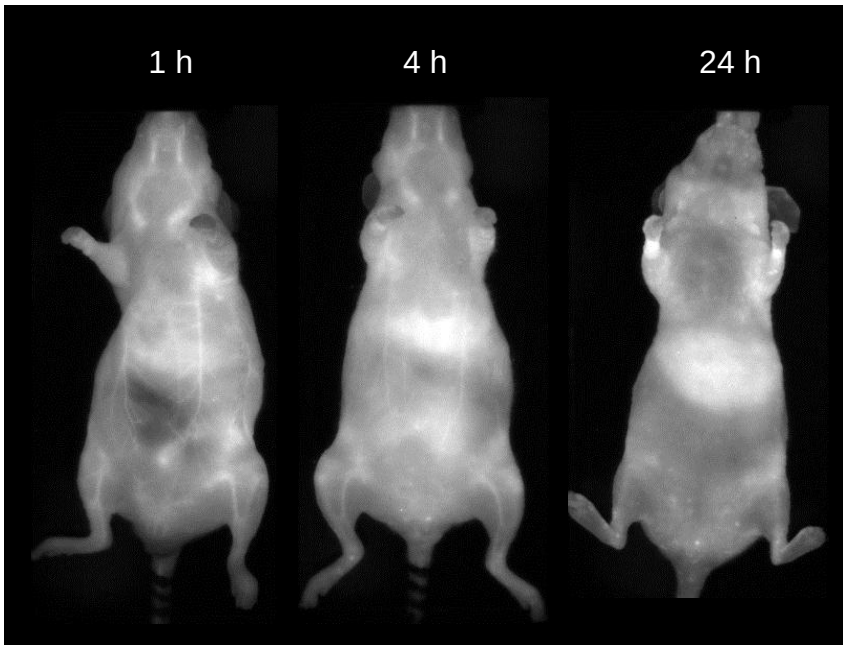


Fig. S3. Whole body fluorescence image of DHLA–PEG2000-capped NIR-QDs.

References

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