Synthesis of cationic quantum dots via two step ligand exchange reaction

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Supporting Information

Materials

All chemicals were purchased from Aldrich unless otherwise stated. The organic solvents were bought from Pharmco-Aaper and used as received while dichloromethane were distilled in the presence of calcium hydride. Flash column chromatography was performed for purification using silica gel (SiO_2, particle size 40-63 μm). Stability studies were performed in Tris buffer (1 M, pH= 8.02), low glucose Dulbecco's Modified Eagle's Medium (Sigma, D5523) and fetal bovine serum (Fisher Scientific, SH3007103).

Synthesis of CdSe/ZnS QDs

CdSe/ZnS core-shell QDs were prepared according to the reported procedure.[i] CdO (0.0514 g, 0.4 mmol), tetradecyl phosphonic acid (TDPA) (0.2232 g, 0.8 mmol) and trioctylphosphine oxide (TOPO) (3.7768 g, 9.77 mmol) were loaded into a 50 ml three-neck flask and heated to 350 ^{o}C under Ar flow. After 3 h the solution becomes optically clear and the Se solution (Se (0.042 g, 0.53 mmol) in 2.4 ml tributyl phosphine (TOP)) was swiftly injected into the hot solution. The CdSe QDs were purified and precipitated with $CHCl_3$ and MeOH, and finally dissolved in $CHCl_3$. Then, the CdSe core solution is mixed with TOPO (4g, 10.3 mmol) and hexadecylamine (HDA) (1.5 g, 6.2 mmol) and heated to 150 ^{o}C for 1 hr. Diethylzinc ($ZnEt_2$) (1.6 ml, 1.6 mmol) in 2.4 ml TOP and and hexamethyl-disilathiane $(TMS)_2S$ (0.278 ml, 1.3 mmol) in 5.25 ml TOP were used as shell solution. After injecting the shell solution the QD mixture was reacted for 1 hr at 100 ^{o}C. The resulting CdSe/ZnS QDs were purified and precipitated with $CHCl_3$ and MeOH, and finally stored in toluene.

Two steps ligand exchange reaction

In first step TOPO/TOP coated QDs (10 mg, 11.45 pmol) were mixed with HS-C5-TEG ligands (30 mg) in MeOH. The reaction mixture was stirred at 35 ^{o}C for 24 h under inert atmosphere. In this step, amphiphilic ligands replaced the native hydrophobic ligands from the surface of QDs, and the resulting amphiphilic QDs became soluble in MeOH. Next step involved the purification of QDs with hexane and addition of dithiol cationic ligands (30 mg) to the amphiphilic QDs in MeOH. As a result, dithiol ligands slowly substitute monothiol ligands from QDs surface due to its better chelating capability

compared to monothiol analogues. After 24 h of stirring, methanol was evaporated and QDs were dispersed in water. The overall yield of this two step ligand exchange reaction was in 96 % (10.98 pmol). Finally the aqueous QDs sample was purified by dialysis.

Electrospray Ionization Mass Spectrometry (ESI-MS)

The mass spectra were acquired at positive mode on a Bruker Esquire-LC (Billerica, MA) quadrupole ion trap mass spectrometer, equipped with an electrospray ionization source. The electrospray needle voltage was set to 3.5 kV, and the capillary temperature was kept as 300 ^{o}C. Usually a voltage of 30 V was applied to skimmer 1 and a voltage of 80 - 90 V was applied to the capillary offset. Samples (~20 μM) were delivered at 200 μL/h using a syringe pump.

Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS)

The MALDI-MS analyses were acquired at positive mode on a Bruker Omniflex time-of-flight mass spectrometer (Omniflex), equipped with a 337 nm nitrogen laser, a 1.0 m flight tube, and a stainless steel sample target. All mass spectra were acquired in reflectron mode. The reflectron voltage was set to 20 kV and the accelerating voltage of 19 kV. On this instrument, an average of 50 laser shots was fired to acquire each spectrum, and a laser power of 10 % was used, which corresponds to approximately 30 μJ/pulse. A saturated α-CHCA stock solution was prepared in 70 % acetonitrile, 30 % H_2O, and 0.1 % trifluoroacetic acid, and to this stock solution was added an equal volume of a 2 μM solution of the QD **1**, QD **2** and QD **3** respectively. 1 μL of this mixture was applied to target, and after allowing it to dry, the MALDI-MS analysis was performed.

TEM

TEM samples were prepared by depositing 5 μL of cationic QDs (5 μM) onto a 300 mesh carbon-coated copper grid. The samples were dried in air at room temperature. TEM images were obtained on a JEOL 100CX electron microscope operated at 100 keV and analyzed using Image J.

DLS and zeta potential

DLS experiments and zeta potential measurements were performed using a Malvern Zetasizer (Nano series, Malvern Instruments Inc, USA). Samples were sonicated and filtered with 0.2 μm syringe filter before measurement.

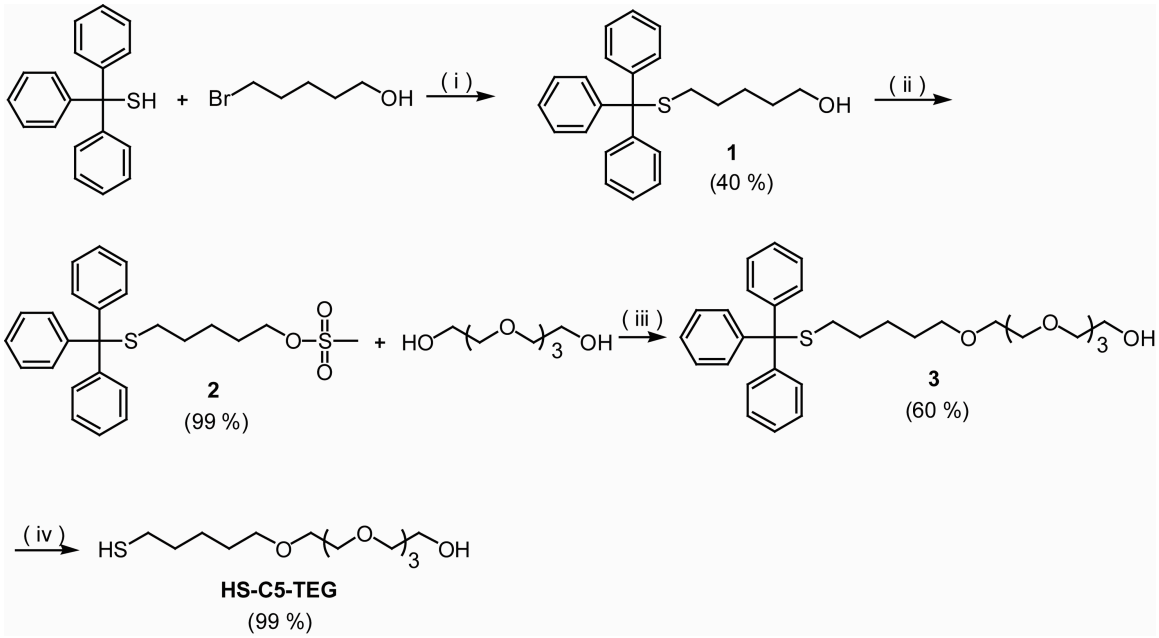
Cell culture

Hela cells were cultured at 37 °C under a humidified atmosphere of 5 % CO_2.The cells were grown in low glucose Dulbecco's Modified Eagle's Medium (DMEM, 4.0 g/L glucose) containing 10 % fetal bovine serum and 1 % antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). The cells were maintained in the above medium and subcultured once in a four days.

Confocal Microscopy

Hela cells were seeded (10×10^4 cells/dish) in a glass-bottom dishes (MatTek Corporation, 14 mm microwell) 24 h prior to the experiment. Next day, old media was removed and cells were washed with cold PBS and 5 nM QD solution in pre-warmed DMEM media (with 10% serum) was added to the cells and incubated for 3 hrs. Thereafter, cells were washed with PBS three times before taking the images. Confocal pictures were obtained on a Zeiss LSM 510 Meta microscope using 63X objective.

Synthesis of HS-C5-TEG ligand



Scheme S1. Synthetic route of the HS-C5-TEG. Reagents and conditions: (i) NaOH, EtOH/toluene, 70 ^oC; (ii) MsCl, NEt_3, DCM, 0 ^oC to r.t.; (iii) NaOH, H_2O/DCM, 90-100 ^oC. (iv) TFA, $(iPr)_3Si$-H, DCM, r.t.

Compound **1**: The mixture of triphenylmethyl mercaptan (9.1 g, 33 mmol) and 5-bromo-pentanol (5 g, 30 mmol) was dissolved in 75 ml of Toluene and 75 ml EtOH. NaOH in 5 ml of H_2O was added. The solution was heated to 70 ^oC and stirred for 6 h. After cooling to r.t. the solution was poured into 300 ml H_2O and neutralized with 1 M HCl. The organic component was washed with H_2O and dried with Na_2SO_4. After evaporation of the solvent, the crude product obtained was purified by column chromatography with 3:1= Hexane/EtOAc as eluent to obtain the Compound **1** in 40 % yield.
^{1}H NMR (400 MHz, CD_3OD): δ 7.44-7.37 (m, 4H, H_{Ar}), 7.36-7.18 (m, 11H, H_{Ar}), 3.54 (t, 2H, CH_2-C<u>H</u>$_2$-OH), 2.15 (t, 2H, -SC<u>H</u>$_2$-), 1.36-1.23 (m, 4H, -C<u>H</u>$_2$-), 0.97-0.79 (m, 2H, -C<u>H</u>$_2$-).

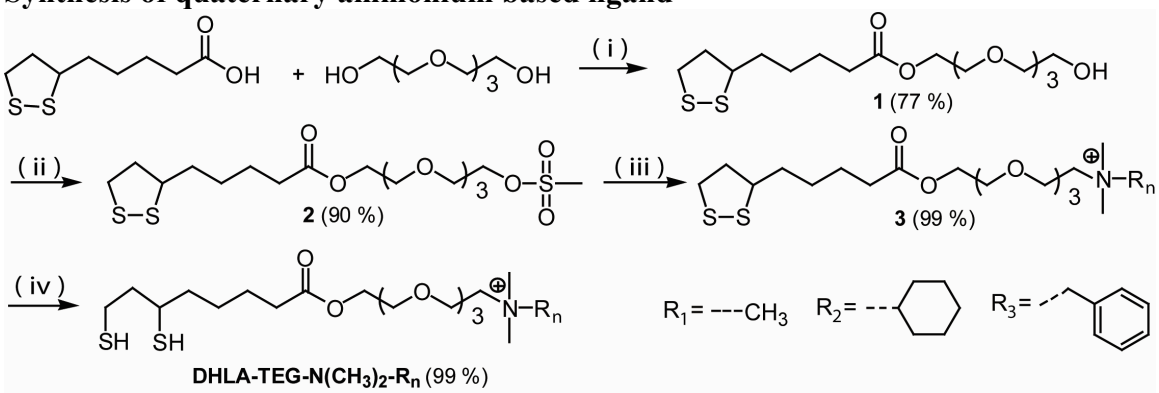
Compound **2**: Compound **1** (4.32g, 12 mmol) was taken in 30 ml of dry DCM, and NEt_3 (3.32 ml, 24 mmol) was added to the solution. The mixture was cooled to 0 ^oC in ice bath. The CH_3SO_2Cl (2.06 g, 18 mmol) was added to the reaction mixture under stirring

condition. The stirring condition was up to 30 min under Ar flow. Then the reaction mixture was allowed to come to r.t. Using TLC to check if the reaction is completed. After completion of the reaction, the reaction mixture was dilutes with DCM and wash with 5 % HCl (twice), $NaHCO_3$ (twice), and water (once). Compound **2** was obtained by using column chromatography with 3:1 = Hexane: EtOAc as eluent in 99 % yield.
^{1}H NMR (400 MHz, CD_3OD): δ 7.46-7.38 (m, 4H, H_{Ar}), 7.34-7.18 (m, 11H, H_{Ar}), 4.12 (t, 2H, CH_2-C<u>H</u>$_2$-OMs), 2.97 (s, 3H, -O-SO_2C<u>H</u>$_3$), 2.15 (t, 2H, -SC<u>H</u>$_2$-), 1.46-1.21 (m, 4H, -C<u>H</u>$_2$-), 0.93-0.81 (m, 2H, -C<u>H</u>$_2$-).

Compound **3**: NaOH (0.75 g, 11.3 mmol) was dissolved in minimum amount of water. Then TEG was added to that aqueous solution under stirring. The solution was heated to 90 – 100 ^{o}C and stirred for 5-10 min. Compound **2** (5 g, 11.3 mmol) was added to the solution under stirring. The reaction mixture was stirred for 24 h at 90 – 100 ^{o}C. Then the reaction mixture was extracted using 25 % EtOAc in hexane. Compound **3** was obtained by using column chromatography with 100 % EtOAc as eluent in 60 % yield. 1H NMR (400 MHz, CD_3OD): δ 7.45-7.38 (m, 4H, H_{Ar}), 7.32-7.16 (m, 11H, H_{Ar}), 3.75-3.51 (m, 14H, -CH_2-*TEG*-), 3.37 (t, 2H, -C<u>H</u>$_2$O-), 2.14 (t, 2H, -SC<u>H</u>$_2$-), 1.51-1.26 (m, 6H, -C<u>H</u>$_2$-).

HS-C5-TEG: Compound **3** (1 g, 1.8 mmol) was dissolved in DCM solution, and TFA (2.73 ml) and (iPr)3Si-H (0.56 ml) was added into the solution. The reaction mixture was stirred for 6 h at r.t. The pure HS-C5-TEG compound was obtained by using column chromatography with 1: 1 = EtOAc: Hxane as eluent in 99 % yield. ^{1}H NMR (400 MHz, CD_3OD): 4.49 (t, 2H, O-CH_2-C<u>H</u>$_2$-OH), 3.79 (t, 2H, CH_2-C<u>H</u>$_2$-O-CH_2), 3.71-3.55 (m, 14H, -CH_2-*TEG*-), 3.46 (t, 2H, -CH_2-C<u>H</u>$_2$O-), 2.52 (t, 2H, -SC<u>H</u>$_2$-), 1.69-1.54 (m, 6H, -C<u>H</u>$_2$-), 1.48-1.38 (m, 2H, -C<u>H</u>$_2$-).

Synthesis of quaternary ammonium based ligand



Scheme S2. Synthetic route of quaternary ammonium based ligand. Reagents and conditions: (i) EDC, HOBt, DIPEA, DCM, r.t., 24 h; (ii) MsCl, NEt_3, DCM, 0 ^{o}C to r.t., 24 h; (iii) $N(CH_3)_2$-R_n/EtOH, 35 ^{o}C, 48 h (iv) $NaBH_4$, EtOH/ H_2O, r.t., 2 h.

Compound **1**: Thioctic acid (TA) (2.297 g, 11.1 mmol) was dissolved in 75 ml dry DCM and cooled to 0 ^{o}C for 5 min. Adding EDC (2.55 g, 13.3 mmol), HOBt (1.7 g, 11.1 mmol), and DIPEA (1.435 g, 11.1 mmol) to the solution, and was purged with Ar gas for several

min. TEG (40 g, 220 mmol) was added, and the reaction mixture was stirred at r.t. for 16 h. Then, the reaction mixture was dilute with DCM, filtered with Celite, and washed with brine and water. Compound **1** was obtained by using column chromatography with 100 % EtOAc as eluent in 77 % yield.
^{1}H NMR (400 MHz, CD_3OD) major peaks assigned: δ 4.24 (t, 2H, COO-C$\underline{H}_2$-CH_2), 3.78-3.64 (m, 12H, -CH_2-*TEG*-), 3.62 (t, 2H, CH_2-C$\underline{H}_2$-OH), 3.22-3.07 (brm, 2H), 2.51-2.42 (brm, 1H), 2.35 (t, 2H, CH_2-C$\underline{H}_2$-COO), 1.76-1.62 (m, 4H), 1.56-1.39 (m, 2H).

Compound **2**: Compound **1** was dissolved in 50 ml of dry DCM, and cooled to 0 ^{o}C in ice bath. NEt_3 and CH_3SO_2Cl was added to the solution under stirring condition at 0 ^{o}C. The reaction was stirred overnight at r.t. Then, the reaction mixture was dilutes with DCM and wash with 5 % HCl, $NaHCO_3$ and water. Compound **2** was obtained by using column chromatography with 1:1 = Hexane: EtOAc as eluent in 90 % yield.
^{1}H NMR (400 MHz, CD_3OD) major peaks assigned: δ 4.38 (t, 2H, COO-C$\underline{H}_2$-CH_2), 4.22 (brs, 2H, -OCH_2-C$\underline{H}_2$-CH_2-OMs), 3.76 (brm, 2H, -CH_2-C$\underline{H}_2$-O-), 3.70-3.63 (m, 10H, -CH_2-*TEG*-), 3.08 (s, 3H, C$\underline{H}_3SO_3^-$), 2.35 (t, 2H, CH_2-C$\underline{H}_2$-COO), 1.71-1.57 (m, 8H), 1.53-1.39 (m, 2H).

Compound **3**: Compound **2** (2 g) was reacted with $N(CH_3)_2$-R/EtOH (3 ml) at 35 ^{o}C for 2 days, and used TLC to check if the reaction is completed. Evaporating the solvent and wash with hexane to obtain the compound **3** in 99 % yield.
^{1}H NMR (400 MHz, CD_3OD) major peaks assigned: δ 4.21 (t, 2H, COO-C$\underline{H}_2$-CH_2), 3.97 (brs, 2H, -OCH_2-C$\underline{H}_2$-CH_2-$N(CH_3)_3$), 3.87 (brm, 2H, COO-CH_2-C$\underline{H}_2$-O), 3.72-3.54 (m, 12H, -CH_2-*TEG*-), 3.38 (s, 9H, -$N(CH_3)_3$), 2.79 (2, 3H, C$\underline{H}_3SO_3^-$), 2.34 (t, 2H, CH_2-C$\underline{H}_2$-COO), 1.75-1.58 (m, 4H), 1.53-1.38 (m, 2H).

DHLA-TEG-N(CH_3)$_2$-R$_n$: Compound **3** (1 g) was dissolved in 20 ml of EtOH / water (1:4) with stirring. $NaBH_4$ (0.15 g) was added and the reaction mixture. The reaction mixture was stirred for 1 hr and become colorless. The reaction mixture was diluted with water and extracted with $CHCl_3$. The combined organic phase was dried over Na_2SO_4. **DHLA-TEG-N(CH_3)$_2$-R$_n$** can be obtained after evaporating the solvent in 99 % yield.

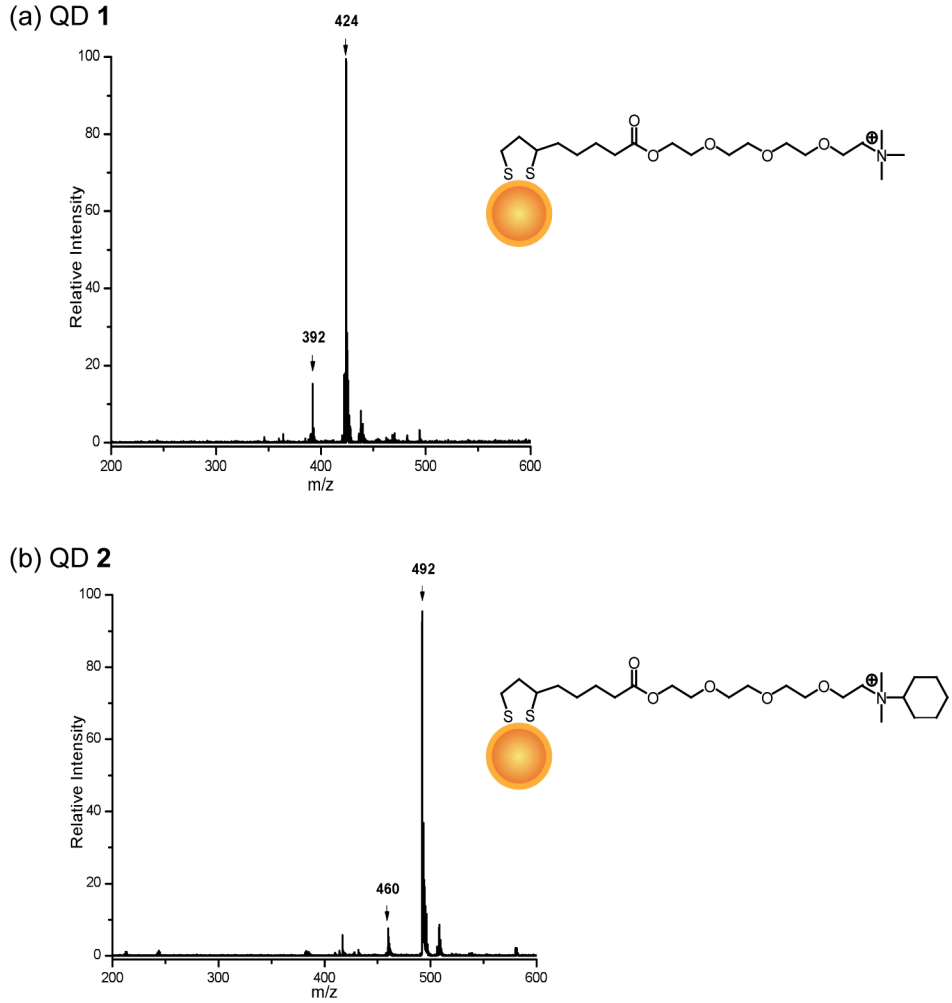
^{1}H NMR and ESI-MS of DHLA-TEG-N(CH_3)$_2$-R$_n$

DHLA-TEG-N(CH_3)$_2$-R$_1$:^{1}H NMR (400 MHz, CD_3OD) major peaks assigned: δ 4.22 (t, 2H, COO-C$\underline{H}_2$-CH_2), 3.98 (brs, 2H, -OCH_2-C$\underline{H}_2$-CH_2-$N(CH_3)_3$), 3.94 (brm, 2H, COO-CH_2-C$\underline{H}_2$-O), 3.71-3.59 (m, 12H, -CH_2-*TEG*-), 3.42 (s, 9H, -$N(CH_3)_3$), 2.35 (t, 2H, CH_2-C$\underline{H}_2$-COO), 1.94-1.86 (m, 2H), 1.8-1.7 (m, 4H), 1.68-1.61 (m, 4H). MS (ESI-MS) calcd for $C_{19}H_{40}NO_5S_2^+$ 426.23, found 426.1 [M].

DHLA-TEG-N(CH_3)$_2$-R$_2$: ^{1}H NMR (400 MHz, CD_3OD) major peaks assigned δ 4.22 (t, 2H, COO-C$\underline{H}_2$-CH_2), 4.00 (brs, 2H, -OCH_2-C$\underline{H}_2$-CH_2-$N(CH_3)_2$-), 3.94 (brm, 2H, COO-CH_2-C$\underline{H}_2$-O), 3.73-3.61 (m, 12H, -CH_2-TEG-), 3.26 (s, 6H, -$N(CH_3)_2$), 2.35 (t, 2H, CH_2-C$\underline{H}_2$-COO), 1.94-1.86 (m, 2H)1.78-1.58 (m, 7H), 1.49-1.36 (m, 6H).
MS (ESI-MS) calcd for $C_{24}H_{48}NO_5S_2^+$ 494.3, found 494.1 [M].

DHLA-TEG-N(CH_3)$_2$-R$_3$: ^{1}H NMR (400 MHz, CD_3OD) major peaks assigned δ 7.64 (m, 2H, HAr), 7.47 (m, 4H, HAr), δ 4.94 (s, 1H, -N(CH_2)$_2$-C$\underline{H}_2$-Ar), 4.89 (s, 1H, -N(CH_2)$_2$-C$\underline{H}_2$-Ar), 4.19 (t, 2H, COO-C$\underline{H}_2$-CH_2), 4.05 (brs, 2H, -OCH_2-C$\underline{H}_2$-CH_2-N(CH_3)$_2$-), 3.93 (brs, 1H, COO-CH_2-C$\underline{H}_2$-O), 3.88 (brs, 1H, COO-CH_2-C$\underline{H}_2$-O) 3.74-3.56 (m, 12H, -CH_2-TEG-), 3.3 (d, 6H, -N(CH_3)$_2$), 2.8 (s, 3H, CH_3-SO_3^--)2.33 (brs, 2H), 2.02-1.81 (brs, 7H), 1.71-1.58 (m, 5H), 1.50-1.37 (m, 3H)
MS (ESI-MS) calculated for $C_{25}H_{44}NO_5S_2^+$ 502.27, found 500.4 [M-2H].

Figure S1 MALDI-MS spectra of QDs



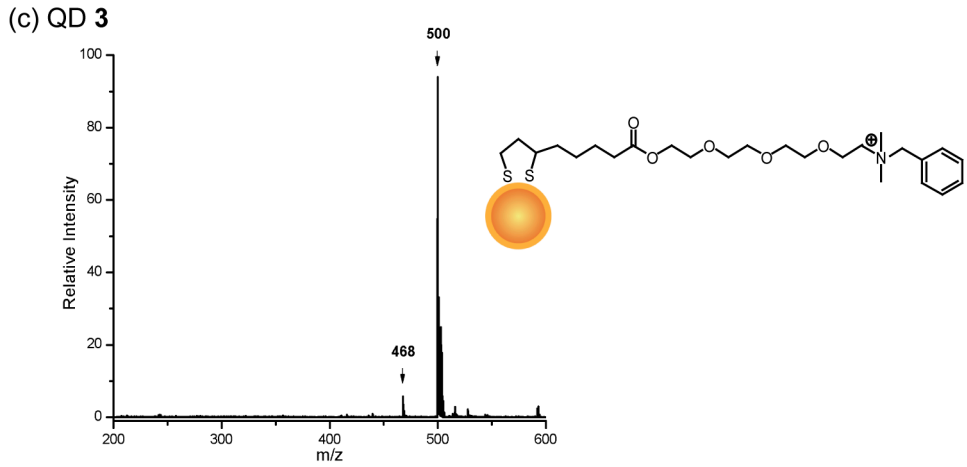


Figure S2 TEM images of (a) TOPO/TOP-QD (b) QD **1**

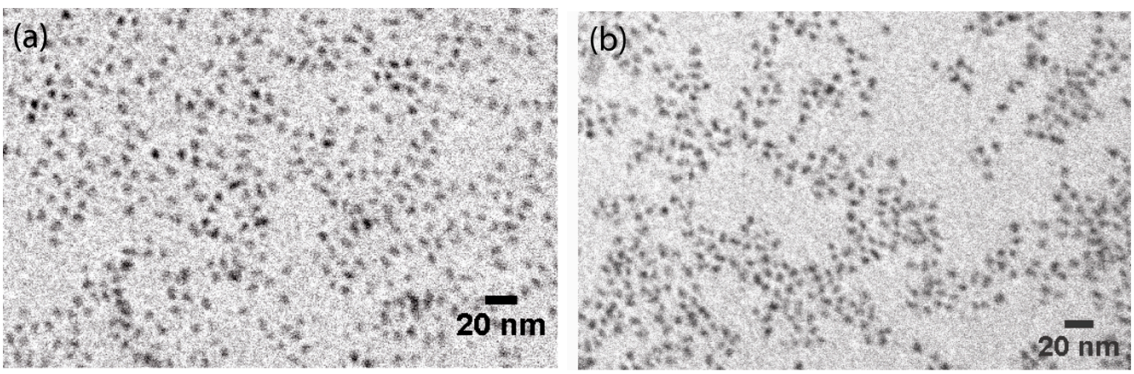
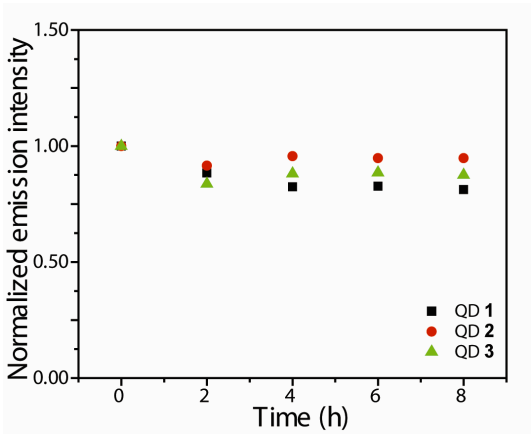


Figure S3 Stability of cationic QDs in saturated NaCl solutions with different QDs: QD **1** (black), QD **2** (red) and QD (green).



Reference:

[i] (*a*) Z. A. Peng, X. G. Peng, *J. Am. Chem. Soc.* 2001, **123**, 183. (*b*) B. O. Dabbousi, J. Rodriguez-Viejo, F. V. Mikulec, J. R. Heine, H. Mattoussi, R. Ober, K. F. Jensen, M. G. Bawendi, *J. Phys. Chem. B* 1997, **101**, 9463.