Supporting Information

Non-invasive Characterization of the Organic Coating of Biocompatible Quantum Dots using NMR Spectroscopy

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Growth of Core-Shell CdSe-ZnS Quantum Dots. The core-shell CdSe-ZnS quantum dots used in this study have been prepared via the "hot injection" method in two steps, following the protocols detailed in previous reports.[1-2] In the first step, CdSe nanocrystals were prepared via reduction of Cd and Se organometallic precursors at high temperature (~350 ˚C) in the presence of surfactants/ligands, namely tri-n-octylphosphine oxide (TOPO), tri-n-octylphosphine (TOP), 1-hexadecylamine (HDA) and n-hexylphosphonic acid (HPA). Briefly, in a typical core growth reaction, 3.125 g TOPO (98%), 1.7 mL TOP (97%), 2.875 g HDA and 0.4 g HPA are mixed in a 50 mL three-neck round-bottom flask equipped with a magnetic stir bar, then heated under inert atmosphere for ~ 2 hours at ~140 ˚C. In a separate container a mixture of 0.3 g Cd(acac)$_2$, 0.57 g HDDO and 2.5 mL TOP (90%) is heated up to 100 ˚C under vacuum for ~ 1 - 1.5 h. The mixture is cooled to 60 ˚C, then 3 mL TOP:Se (1 M) solution is added. The atmosphere in the three-neck flask is switched to nitrogen, and the temperature is increased to 340 - 350 ˚C. Once the target temperature is reached, the mixture of precursors is rapidly injected into the flask. Growth and annealing is then applied as described in references.[1-2] The grown core nanocrystals are then purified and prepared for the over-coating reaction. In the second step, the CdSe cores are overcoated with few monolayers of ZnS using relatively lower temperature (150~180 ˚C). The purified core materials are dispersed into TOPO (90%), then a mixture of diethylzinc (ZnEt$_2$), hexamethyldisilathiane ((TMS)$_2$S) and TOP (90%) is added dropwise to target 5~6 monolayers of ZnS overcoating, following the protocol described in reference.[2] As-prepared the CdSe-ZnS QDs are hydrophobic in nature and usually stored at room temperature in dispersions of toluene/hexane/butanol mixture containing excess ligands for further use. The QD concentration is extracted from the absorbance at 350 nm using the Beer-Lambert law and the molar absorption coefficient associated with the particular core size. The latter can be extracted following the procedure reported by Bawendi and co-workers.[3] The TEM images collected from the QDs shown in Figures S7 and S8 indicate that homogeneous nanocrystals are prepared.

Aqueous Phase Transfer of QDs Promoted by Photoligation. Phase transfer based on ligand exchange with LA-based ligands was implemented using a photoligation strategy developed by our group.[4] Briefly, a stock solution of hydrophobic CdSe-ZnS QDs (1 mL, concentration ~ 1-5 µM) was precipitated using 10 mL of ethanol, centrifuged at 3,600 RPM for 5 min and the

supernatant was discarded. The precipitate/pallet was redispersed in hexane (500 μL), and then mixed with a solution of the ligand (**L1**, **L2**, or **L3**) in methanol (500 μL). The mixture QD-plus-ligands was placed in a UV photo-reactor and irradiated with continuous stirring for 30-40 min. The UV photoreactor used (Luzchem UV lamp, model LZC 4V) has 14 lamps, six installed on top and four on each side, generates a broad signal centered at 350 nm and a power of 4.5 mW/cm^2 at the sample position. Transfer of QDs from the hexane to the methanol layer is usually taken as a sign that ligand exchange with LA-PEG-OCH$_3$ has taken place.[4] Once the ligand exchange process was complete, the solvent was removed under vacuum. A solvent mixture (1:1:10, methanol: chloroform: hexane) was added to the dry sample, the mixture was sonicated and then centrifuged at 3,600 RPM for 5 min, yielding a precipitate. The supernatant was discarded, the QD pellet was dried under vacuum, and then dispersed in DI water. The same steps have been applied to all three sets of QDs and three types of ligands used.

Dynamic Light Scattering Measurements. The DLS data were collected using an ALV/CGS-3 Compact Goniometer System equipped with a He-Ne laser source (λ = 633 nm), an avalanche photodiode for signal detection and an ALV-7004 correlator (ALV-GmbH, Langen, Germany). The scattered signal was collected at 90 degrees and consisted of an average of 3 acquisition periods of 10 seconds each. The auto-correlation function was fitted to a cumulant series using ALV software to yield a measure of the diffusion coefficient, D.[5-6] From this value we extract a measure for the hydrodynamic radius (R$_H$) of the QDs using the Stokes-Einstein relation:

$$R_H = \frac{kT}{6\pi\eta D} \qquad (1)$$

where k is the Boltzmann constant, T the temperature in Kelvin, and η is the solvent viscosity. The concentrations used to collect the DLS profiles were ~0.2 μM.

Transmission Electron Microcopy (TEM). The TEM images of the QDs before and after ligand exchange and phase transfer were collected using a JEM-ARM200CF (A Sub-Angström Cs Corrected Transmission/Scanning Transmission Electron Microscope from JEOL) operated at 200 kV, available at the FSU National Magnetic Laboratory. The TEM grid were prepared by drop casting dilute dispersions of QDs in either toluene or water followed by drying.

Table S1: Estimates of the number of ligands per QD, ligand density and foot print area (FPA) associated with each QD-plus-ligand set, using sodium benzoate as the standard.

QD set	Ligand	Total Number of Ligands [@]	Ligand Density (nm^{-2})	FPA (nm^2)
Green QDs (λ_{em} = 540 nm) (radius $\cong$ 3 nm)	LA-PEG-OCH$_3$, **L1**	163	1.44	0.69
	LA-(PEG-OCH$_3$)$_2$, **L2**	80	0.71	1.41
	bis(LA)-PEG-OCH$_3$, **L3**	98	0.87	1.15
Yellow QDs (λ_{em} = 570 nm) (radius $\cong$ 3.4)	LA-PEG-OCH$_3$, **L1**	277	1.91	0.52
	LA-(PEG-OCH$_3$)$_2$, **L2**	150	1.03	0.97
	bis(LA)-PEG-OCH$_3$, **L3**	183	1.26	0.79
Orange QDs (λ_{em} = 590 nm) (radius $\cong$ 3.6 nm)	LA-PEG-OCH$_3$, **L1**	376	2.31	0.43
	LA-(PEG-OCH$_3$)$_2$, **L2**	240	1.47	0.68
	bis(LA)-PEG-OCH$_3$, **L3**	312	1.91	0.52

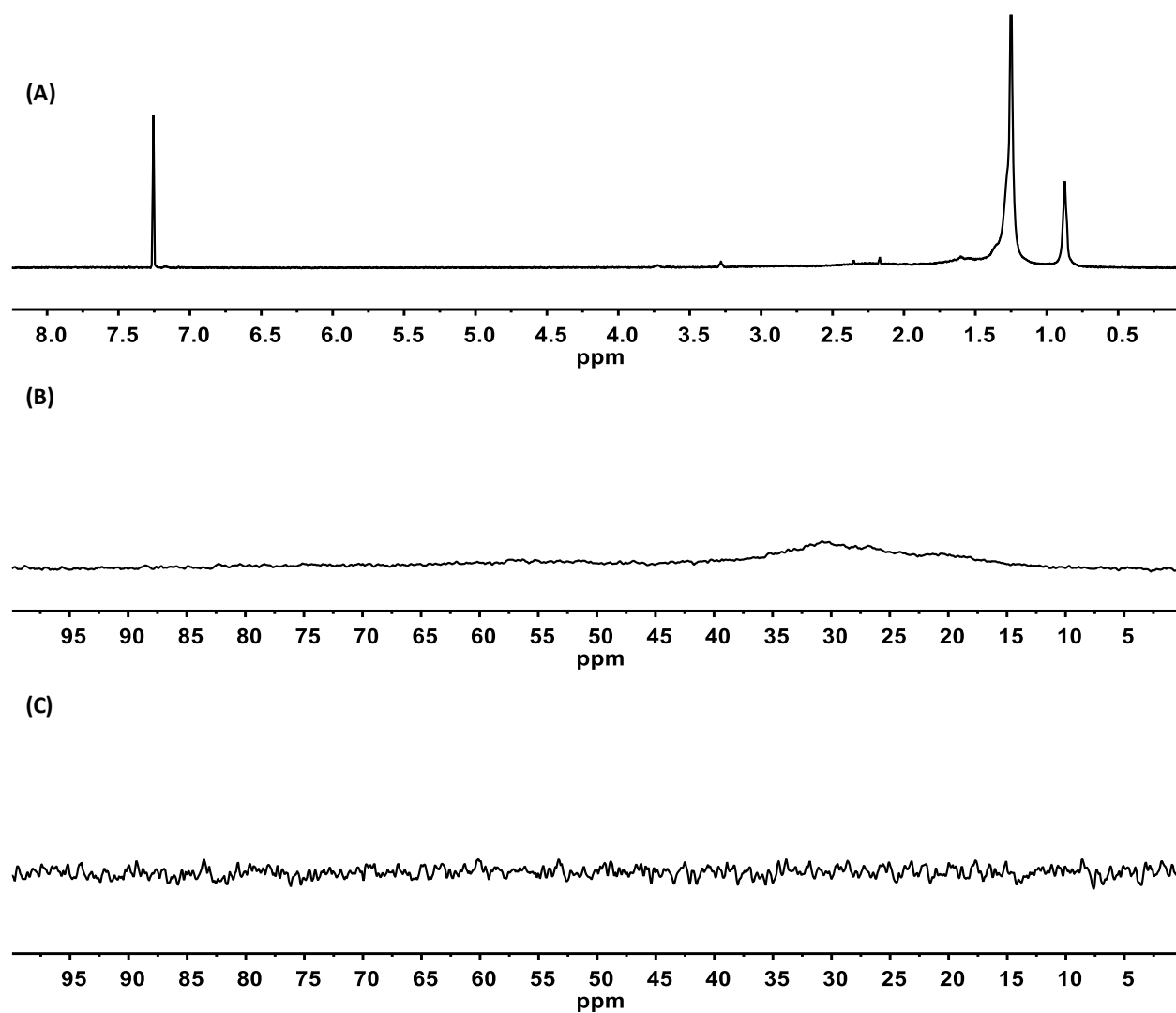[@] extracted from averaging over two measurements.

Figure S1: (A) ^{1}H NMR spectrum collected from a hydrophobic QD dispersion (i.e., QDs capped with TOPO/TOP/HDA) after two rounds of purification then dispersed in chloroform-d. (B and C) ^{31}P NMR spectra of hydrophilic LA-PEG-OCH$_3$-capped QDs after two and three rounds of purification, respectively, then dispersed in D$_2$O. The phosphonate signature, expected at ~ 27 ppm, has disappeared after 3 rounds of purification.
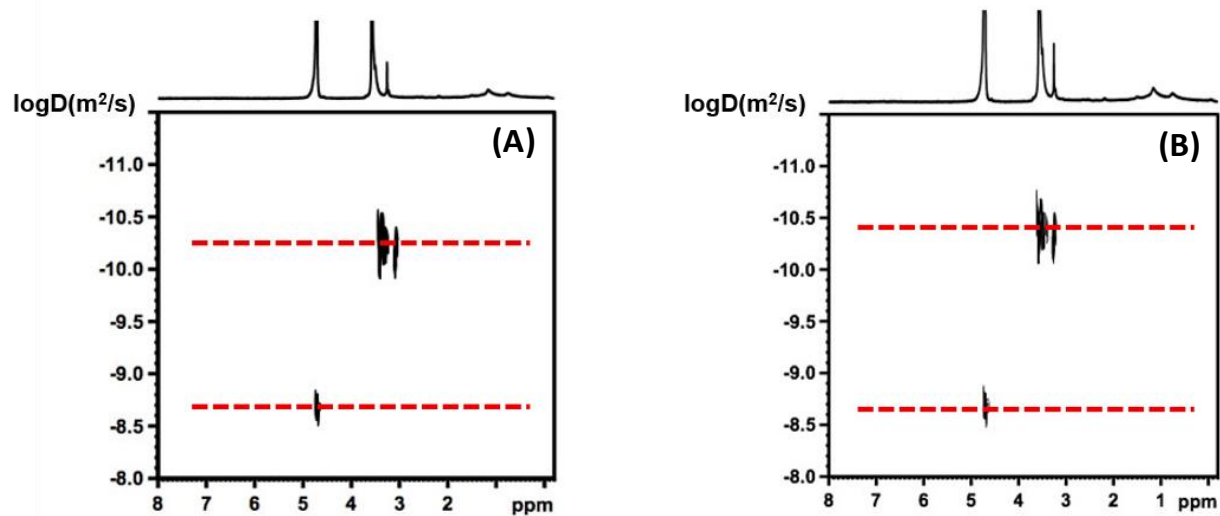
Figure S2: DOSY NMR spectra of photoligated bis(LA)-PEG-OCH$_3$-QDs (A) and LA-(PEG-OCH$_3$)$_2$-QDs (B), after three rounds of purification and then dispersed in D$_2$O. Yellow-emitting QDs were used.
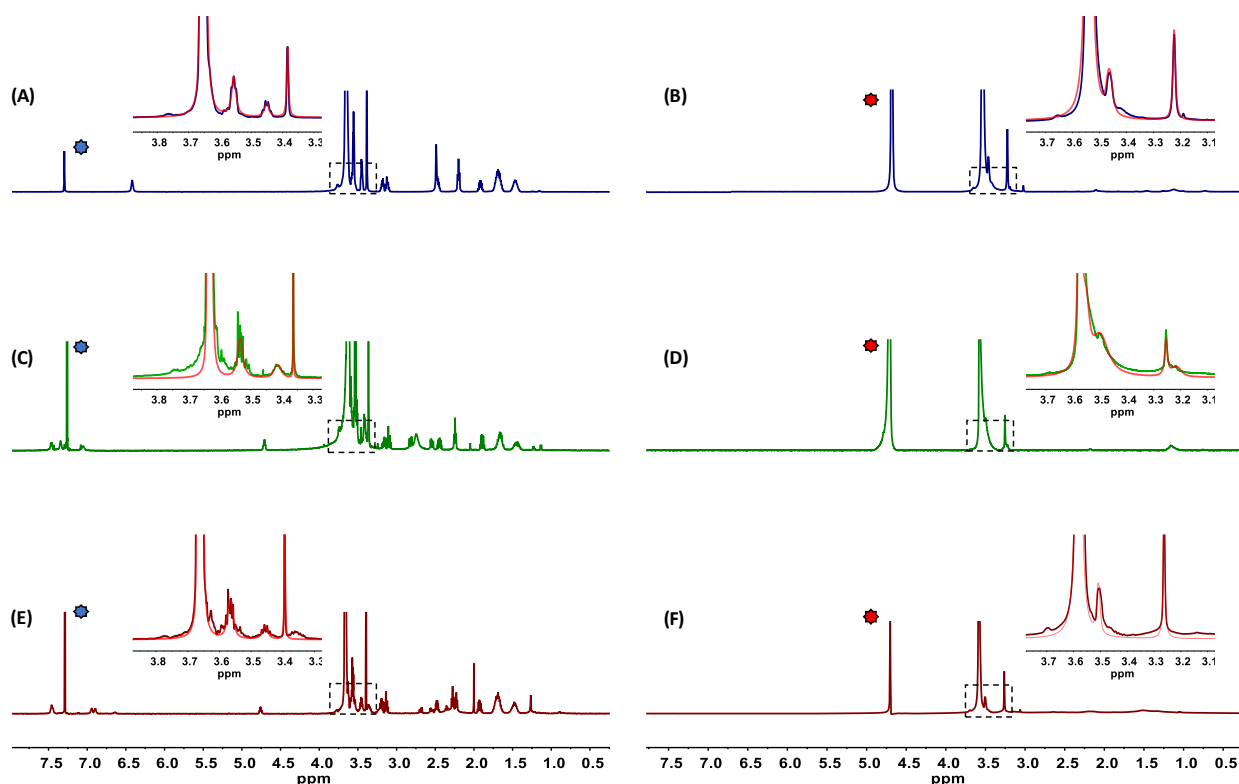
Figure S3: Duplicate of the data shown in Figure 3 with Gaussian line fittings included (orange lines in all graphs). (Left panels) ^{1}H NMR spectra of (A) LA-PEG-OCH$_3$, (C) LA-(PEG-OCH$_3$)$_2$, (E) bis(LA)-PEG-OCH$_3$ in chloroform-d solutions. (Right panels) ^{1}H NMR spectra of (B) LA-PEG-OCH$_3$-QDs, (D) LA-(PEG-OCH$_3$)$_2$-QDs, (F) bis(LA)-PEG-OCH$_3$-QDs dispersed in D$_2$O. All three QD dispersions were subjected to three rounds of purification prior to collecting the spectra. Yellow-emitting QDs have been used for these measurements. The labels in the left and right panels designate the solvent peak (CHCl$_3$ for the pure ligand and H$_2$O for the dispersions). Also visible in the ligand spectra (~4.75 ppm, C and E) is the signature of the alpha proton in the aspartic acid.
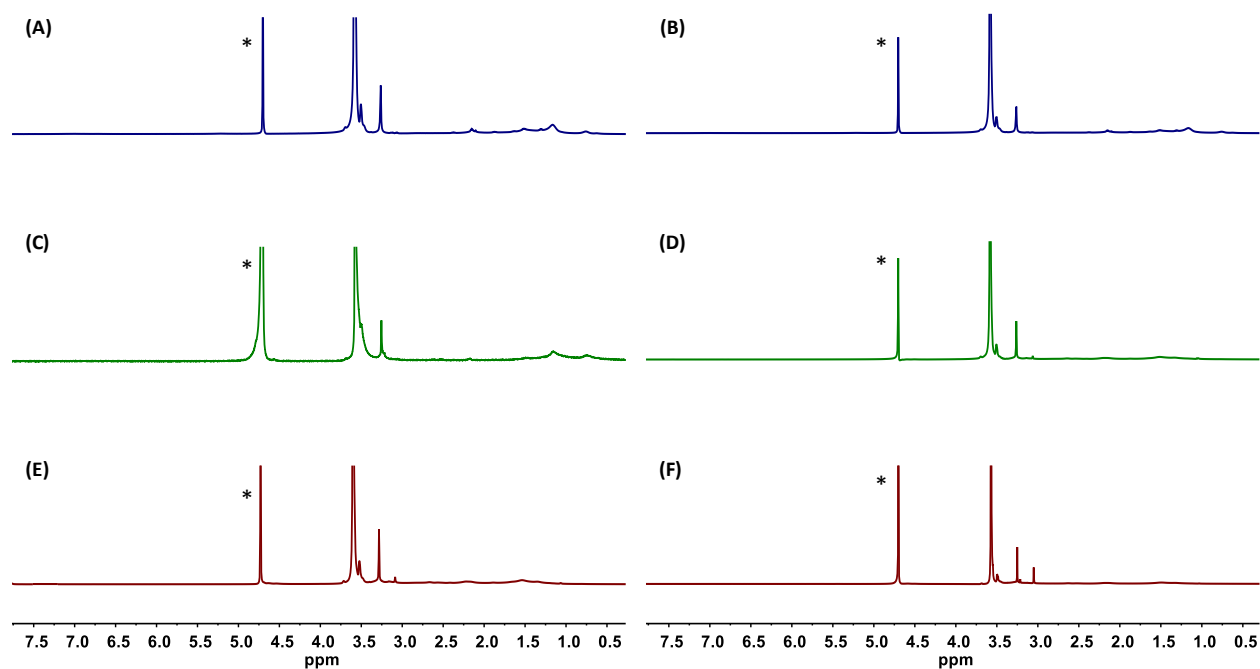
Figure S4. (Left panels) ^{1}H NMR spectra of green-emitting QDs capped with (A) LA-PEG-OCH$_3$, (C) LA-(PEG-OCH$_3$)$_2$, and (E) bis(LA)-PEG-OCH$_3$. (Right panels) Similar spectra collected from orange-emitting QDs capped with (B) LA-PEG-OCH$_3$, (D) LA-(PEG-OCH$_3$)$_2$, (F) bis(LA)-PEG-OCH$_3$. The spectra were collected without applying water suppression; the black stars designate water impurities. The samples have been subjected to three rounds of purification, and then dispersed in D$_2$O.
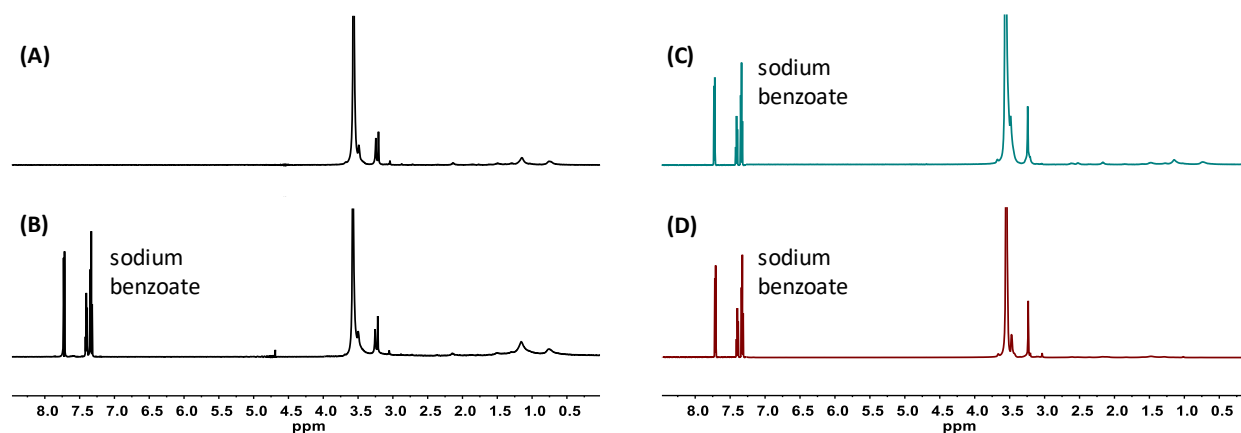
Figure S5: ^{1}H NMR spectra of: (A) LA-PEG-OCH$_3$-QD dispersion; (B) the same dispersion of QDs mixed with sodium benzoate (external standard) in D$_2$O; (C) LA-(PEG-OCH$_3$)$_2$-QD dispersion mixed with sodium benzoate; (D) bis(LA)-PEG-OCH$_3$-QDs dispersion mixed with sodium benzoate. Water suppression was applied to all four collected spectra. The concentration of added standard was not identical for all shown spectra. Note that in panels A and B the shifts associated with the -OCH$_3$ and the -CONH-CH$_2$ are close but do not completely merge, which is occasionally observed for certain QD samples. All samples were subjected to three rounds of purification prior to collecting the spectra.
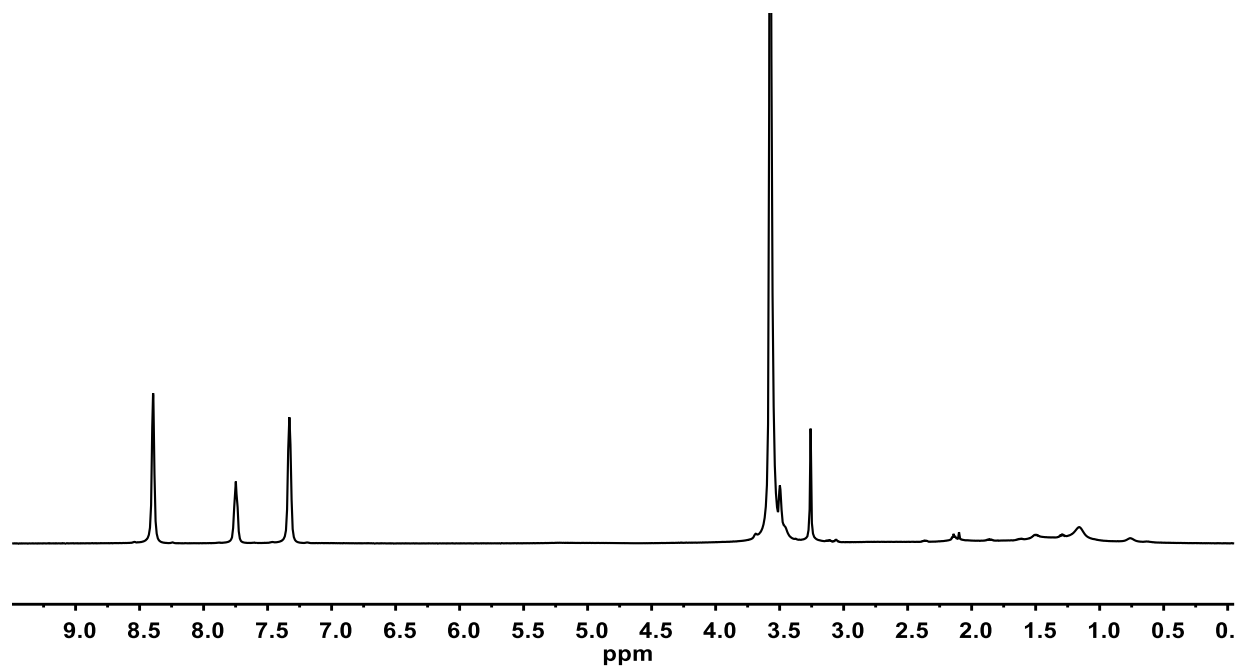
Figure S6: ^{1}H NMR spectrum of LA-PEG-OCH$_3$-QDs subjected to three rounds of purification, dispersed in D$_2$O and then mixed with a known concentration of pyridine (as external standard); the pyridine signature spans over the range 7.25 – 8.5 ppm.
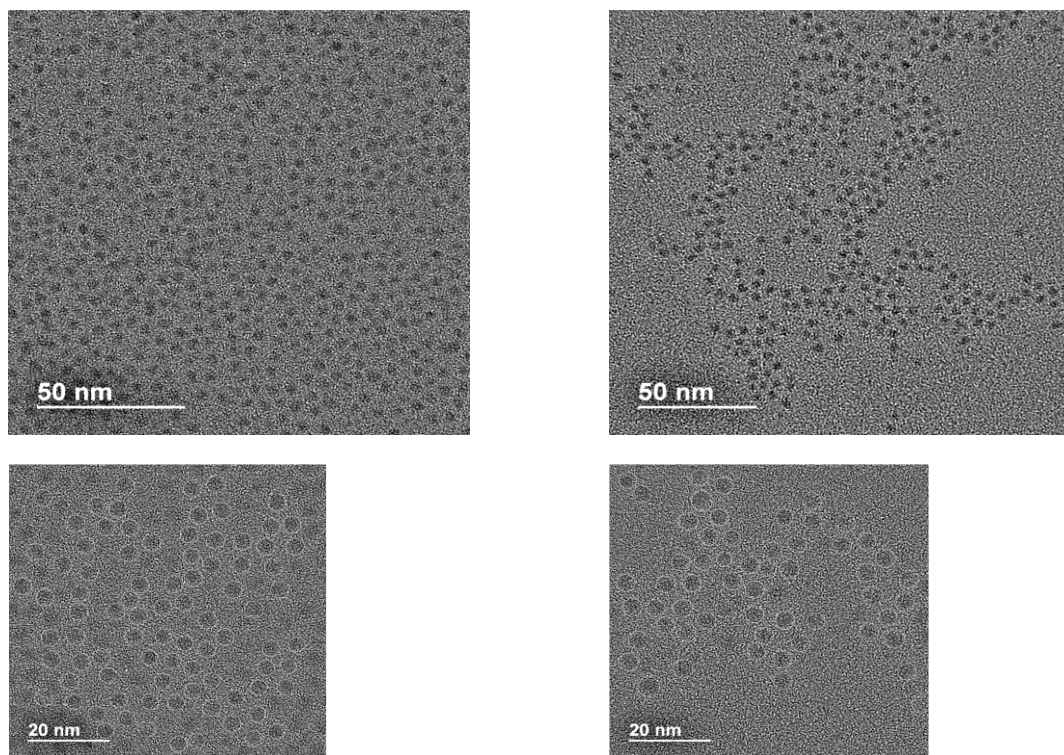
Figure S7: High-resolution TEM images collected from hydrophobic green- (left images) and yellow- (right images) emitting core-shell QDs. The white circles delineate the edges of the inorganic cores of individual QDs.
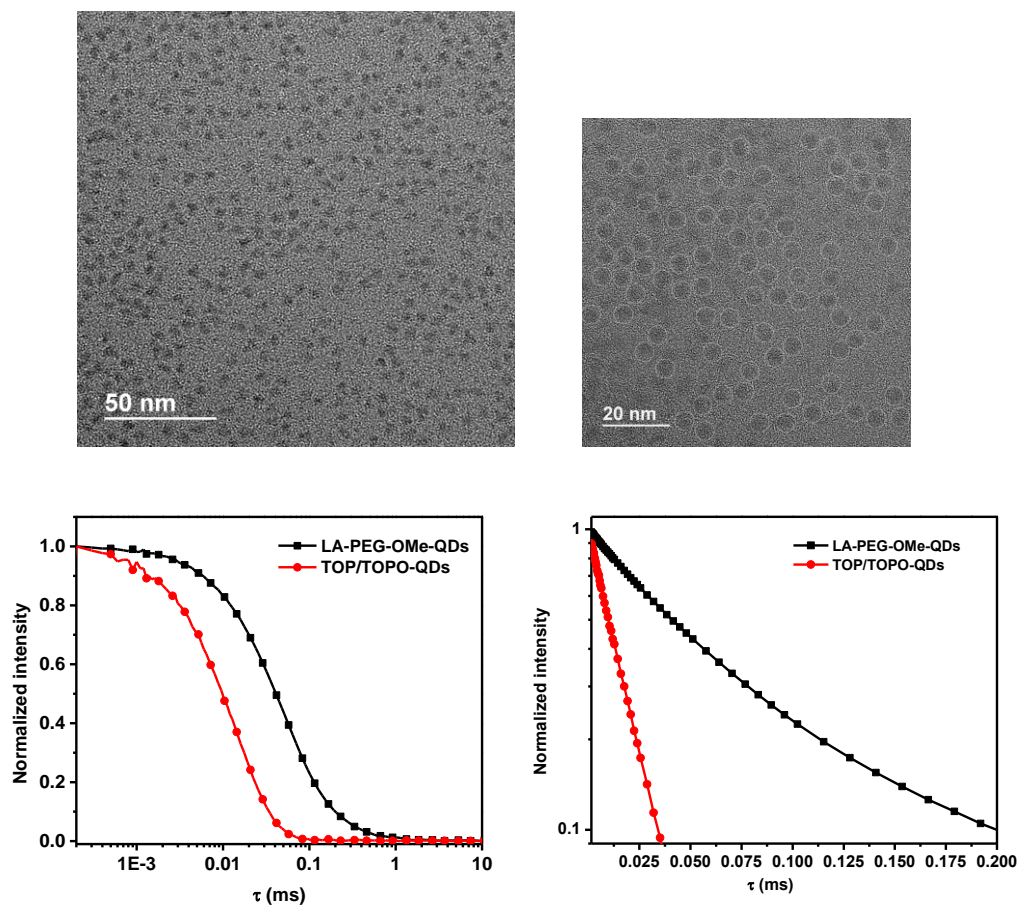
Figure S8: (Top panels) High-resolution TEM images collected from yellow-emitting QDs after phase transfer. (Bottom panels) Dynamic light scattering data collected from yellow-emitting QDs before and after phase transfer. Shown are plots of the intensity auto-correlation function, $g^{(2)}(\tau)$ vs log(τ) and log($g^{(2)}$) vs τ. Those profiles were collected at a scattering angle θ = 90 degrees. The measured diffusion coef., D, and the corresponding hydrodynamic radius, R_H, are :D = $8.3\times10^{-7}cm^2s^{-1}$ and R_H = 4.4 nm (for TOP/TOPO-QDs); D = $2.7\times10^{-7}cm^2s^{-1}$ and R_H = 8.4 nm (for LA-PEG-OMe-QDs). The increase in hydrodynamic size measured for the hydrophilic QDs is expected due to the larger spatial extension of the PEG ligands.

References

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