Quantitative Characterization of the Lipid Encapsulation of Quantum Dots for Biomedical Applications

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Chemicals

n-Hexadecylamine (HDA, 90%), trioctylphosphine oxide (TOPO, 90%), trioctylphosphine (TOP, 90%), tributylphosphine (TBP, 97%), stearic acid (SA, 95%), octadecylamine (ODA, 99%) and 1-dodecanethiol (98%) were purchased from Sigma Aldrich (St. Louis, Missouri) and used without further purification. The precursors CdO (99.95%), Se (99.99%), $Cd(C_2H_3O_2)_2 \cdot 2H_2O$ (98%), $Zn(C_{18}H_{35}O_2)_2$ (Tech Grade), and bis(trimethylsilyl) sulfide ($(TMS)_2S$, purum) were purchased from Sigma Aldrich. 1-myristoyl-2-hydroxy-sn-glycero-3-phosphocholine (MHPC), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N- [methoxy (polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG2k), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE), 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-N-(lauroyl) (sodium salt) (DPPE-COOH), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-(lauroylamine) (DPPE-NH$_2$) were purchased from Avanti Polar Lipids (Alabaster, Alabama). Hexane, methanol, chloroform and ethanol were HPLC grade.

Synthesis of CdSe QDs

Synthesis of CdSe/(Cd,Zn)S QDs.

CdSe cores were synthesized from CdO and Se in TOPO and HDA. In a 250 mL reaction vessel, 2.35 mmol CdO was dissolved in 4.0 g of stearic acid (14.1 mmol) by heating to 180 °C under argon. Upon the dissolution of CdO to form a homogenous, light yellow solution, the heat was removed and the reaction vessel cooled to 80 °C. The reaction vessel was opened under argon and 10.8 g HDA (44.7 mmol) and 4.8 g TOPO (12.4 mmol) were added. After degassing under vacuum at 135 °C for one hour, the reaction vessel was heated to 300 °C under argon. In an argon glove box, 11.5 mmol Se was vigorously mixed with 4 mL TBP (16.1 mmol) and loaded

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into a syringe for injection. When the temperature reached 300 °C, the TBP/Se precursor was rapidly injected into the reaction mixture, resulting in a total volume of about 25 mL. After 10 min, the heat was removed and the reaction vessel was allowed to cool. When the temperature reached 130 °C (7 - 10 min), 25 mL hexane was slowly injected into the reaction mixture to prevent solidification of the TOPO/HDA. The warm, viscous reaction mixture was quickly transferred into two 50 mL conical tubes and 25 more mL of room temperature hexane was added filling each tube. The reaction slurry was centrifuged at 2500 rpm for 1 min and the supernatant was transferred to two new conical tubes. The slurry was centrifuged again at 2500 rpm for 3 min and the precipitation discarded. Finally, the supernatant is centrifuged at 4000 rpm for 4 min and the precipitation discarded. Once CdSe is removed from excess TOPO/HDA/SA, it is precipitated out of hexane using 25 mL methanol and centrifugation at 4000 rpm for 5 min. The supernatant was discarded and the precipitated QDs were dried and resuspended in 6 mL hexane. The QDs were transferred from a 50 mL conical to a 15 mL conical tube for centrifugation at 8000 rpm for 10 min. This effectively removes all remaining insoluble material. To the remaining supernatant, 5 mL methanol was added for one final precipitation at 8000 rpm 5 min. After weighing, the final precipitant was resuspended in 3 mL hexane.

The precursor concentrations for the (Cd,Zn)S shell were calculated based upon the formation of 3.5 monolayers composed of 70 mol% Zn and 30 mol% Cd on 80 mg of 6.0 nm diameter CdSe QDs. 10 g TOPO (26 mmol) and 6.0 g HDA (25 mmol) were degassed for one hour at 100 °C. Next, 80 mg CdSe in hexane was injected under argon and the flask quickly returned to vacuum. The 0.2 M zinc/cadmium precursor was prepared by combining 0.410 g zinc stearate (0.64 mmol) in 3.2 mL toluene and 0.074 g $Cd(C_2H_3O_2)_2 \cdot 2H_2O$ (0.in 1.4 mL TOP under argon at 110 °C in a sealed 20 mL glass for one hour. The sulfur precursor was prepared in the argon glovebox by combining 2 mL TOP with 0.4 mL $(TMS)_2S$ under nitrogen such that the molar ratio of zinc/cadmium to sulfur is 1:2. After two additional hours of degassing, the flask was switched to argon and the temperature raised to 220 °C for injection. The Cd/Zn and sulfur precursors were slowly injected over 55 min using separate syringe pumps. When the injection was completed the reaction mixture was held at 240 °C for 15 min at which point the heating mantle was removed and the flask was allowed to cool to 110 °C. To extract the QDs, 25 mL methanol was injected to prevent solidification of HDA/TOPO. The 50 mL reaction

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mixture was transferred to a conical tube and centrifuged at 2500 rpm for 2 min, the supernatant discarded, and the precipitated QDs re-suspended in 5 mL hexane. The slightly cloudy suspension was transferred to a 15 mL conical tube, centrifuged twice (8500 rpm for 5 min) and the precipitant discarded producing a final homogeneous suspension of QDs in hexane. The QDs were transferred to chloroform by precipitating with methanol, dried under vacuum, and re-suspended in 4 mL of chloroform at a concentration 30 to 40 mg mL^{-1}.

CdSe(Cd,Zn)S thiolation.

The amount of thiol needed to displace the HDA/ODA ligands was calculated from the footprint for 1-dodecanethiol (0.21 nm^2)[1] and the total surface area of the QDs. The total surface area of the CdSe/(Zn,Cd)S QDs was estimated from the concentration using a diameter of 8.0 nm. A seventy-fold excess of thiol was added to the QDs. In a 7 mL borosilicate glass vial, 400 μL of QDs (10 nmole) in chloroform was combined with 130 μL of DDT (0.54 mmole). The vial was sealed and mixed on a hotplate at 75 °C with vigorous stirring overnight. The unreacted thiol was removed through three precipitations and resuspensions. The mixture was combined with 100 μL chloroform, 500 μL ethanol and 100 μL methanol in a 1.7 mL conical vial and centrifuged at 9000 rpm for 3 min. The QDs were resuspended in 400 μL chloroform using vortexing and sonication and precipitated again with 800 μL ethanol at 9000 rpm for 4 min. The precipitant was resuspended again in 400 μL chloroform and precipitated again with ethanol centrifuging for 4 min at 9000 rpm. The precipitant was dried for 30 s under vacuum and resuspended in 400 μL chloroform.

Characterization

QY was measured by mixing 4 mL double distilled water with between 30 μL of QD suspension at high fractions recovered to as much as 150 μL of QD suspension at lower fractions recovered. Particle size distributions and zeta potential were obtained for QD concentrations between 1.0 x 10^{-7} and 2.3 x 10^{-7} mol L^{-1} with a volume of 0.4 mL (size distribution) or 0.7 mL (zeta potential) using a Nano Zetasizer (Malvern, Worcestershire, UK). In both cases the measurement protocol was set manually to five runs (size distribution) or six runs (zeta potential) with automatically determined sub-runs and no equilibration time. The particle size distributions and zeta potential were determined after discarding the first run.
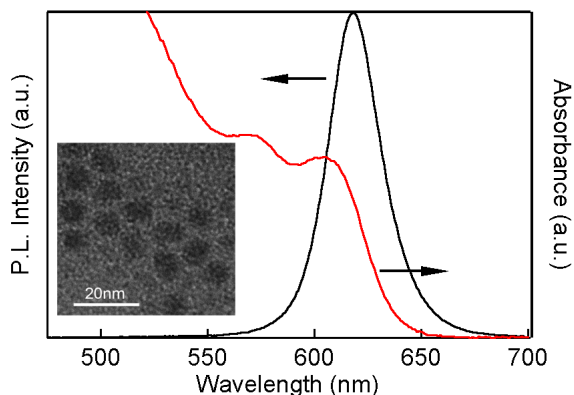
Core/shell QDs

Figure S1 shows representative photoluminescence and absorbance spectra for CdSe/(Zn,Cd)S QDs in chloroform after synthesis. The PL peak after capping is at 618 nm with a full width at half maximum (FWHM) of approximately 28 nm. The absorbance curve shows an onset at about 650 nm and a first exciton peak at 604 nm. The sharp absorbance edge and narrow PL indicate a narrow size distribution. The inset shows a representative TEM image showing nearly spherical CdSe/(Cd,Zn)S QDs with an average size of 7.9 ± 0.6 nm. The measured QY of the QDs in chloroform was 71%. These results are typical of the QDs used for water solubilization in this study.

Lipid Coating

SA and MHPC water solubilization. The total amount of lipids needed to functionalize the QDs was determined from the total surface area and the footprint of MHPC and SA. The radius of the CdSe/(Zn,Cd)S QDs with an HDA/TOPO or DDT inner leaflet was taken as 4.0 nm. The footprints for MHPC and SA were taken to be 0.5 nm^2 and 0.2 nm^2, respectively.[2, 3] The volume of QDs used for solubilization was based on achieving a final concentration of QDs in water of 2.5×10^{-7} M. To ensure successful water solubilization, a 7 fold-excess of lipid was used for all experiments. SA was freshly dissolved in chloroform to give a solution of 25 mg mL^{-1} and then sonicated to ensure complete mixing. MHPC was warmed from 20 °C to room temperature and then sonicated to obtain a homogenous solution. The QD suspension, MHPC solution, and SA solution were mixed by vigorous pipetting and then sonicated in a water bath for 20 s. Immediately after sonication, the QDs/MHPC/SA solution was added dropwise to 2 mL Millipore water in a 7 mL borosilicate glass vial under vigorous stirring using a 3 x 13 mm stir

bar at maximum speed. Once completely mixed, approximately one minute, the temperature of the hot plate was raised to 90 °C. After 45 - 50 min, a near homogenous solution is formed with color ranging from bright orange to green orange. The vial was sonicated in a water bath for 90 min. Upon completion of sonication, the solution was transferred to a centrifuge tube and spun at 8000 rpm for 3 min. The supernatant was removed using a syringe and filtered through a 200 nm, PTFE 13 mm diameter syringe filter. The range of concentrations collected after QD water solubilization varied between approximately 2.5×10^{-8} M up to 2.3×10^{-7} M.

ODA and MHPC water solubilization. ODA-functionalized QDs were prepared in the same way as described for SA-functionalized QDs. For ODA functionalization, a 25 mg mL^{-1} solution in chloroform was freshly prepared and then sonicated to ensure complete mixing. The footprint of ODA is taken as 0.2 nm^2.[4] Water solubilization using ODA was then carried out as described above for SA.

DSPE and MHPC water solubilization. The water solubilization of QDs using a combination of single and double acyl chain lipids was similar to the method described above for SA and ODA with slight modifications. The footprint of the double acyl chain phospholipid DSPE was taken to be 0.55 nm^2.[5] The QD suspension, DSPE solution, and MHPC solution were combined in a vial, mixed thoroughly by pipetting, and sonicated for 20 s. Immediately after sonication, the mixture was added dropwise to Millipore water under vigorous stirring. After 1 minute, the hotplate temperature was raised to 110 °C. At one hour, the slightly turbid mixture was transferred to the sonicator for 1 h. A near homogenous solution was transferred to a centrifugal tube and spun at 8000 rpm for 3 min. The supernatant was then filtered through a 200 nm, PTFE 13 mm diameter syringe filter.

Functionalized Lipids and MHPC water solubilization. To investigate the influence of functional groups we studied the incorporation of DPPE-COOH and DPPE-NH$_2$. The total percentage of double acyl chain phospholipids (DPPE-COOH or DPPE-NH$_2$ and DPPE) in the bulk solution was fixed at 20 mol% with 80 mol% MHPC. The MHPC, DPPE and DPPE-COOH or DPPE-NH$_2$ lipids were combined with the QDs in chloroform in a small vial and mixed by pipetting and sonication for 20 s. After sonication, the lipids and QDs were added to double-distilled water under vigorous stirring. After 1 minute the temperature was increased to 90°C and chloroform allowed to evaporate for 45 - 50 min. Next, the samples were transferred

to the sonicator for 90 min. When sonication was completed, the samples were centrifuged at 8000 rpm for 3 min and then filtered through a 200 nm, PTFE 13 mm diameter syringe filter.

DSPE-PEG and MHPC water solubilization. DSPE-PEG functionalized QDs were prepared in the same way as described above for DSPE QDs. The footprint of DSPE-PEG was taken to be the same as for DSPE (0.55 nm^2).[5]

ODA and DSPE-PEG2k with MHPC. The solubilization of QDs using a three-component lipid layer was performed in a similar way to the ODA and DSPE-PEG2k functionalized QDs with slight modifications. A 25 mg mL^{-1} solution of ODA in chloroform was freshly prepared and sonicated for 5 min. Solutions of DSPE-PEG2k and MHPC in chloroform were sonicated for 5 min. The QD suspension, ODA, DSPE-PEG2k, and MHPC solution were added to a vial and mixed by pipetting and sonicating for 20 s. The sonicated mixture was added dropwise to 2 mL Millipore water in a 7 mL vial on a hot plate under vigorous stirring. After 1 minute the temperature was raised to 95 °C and stirring continued for 50 min at which point the vial was transferred to the sonicator for 90 min. At the conclusion of sonication, the QDs were centrifuged at 8000 rpm for 3 min and filtered through a 200 nm, PTFE 13 mm diameter syringe filter.

Relationship between conductivity and ionic strength

To relate the measured conductivity (S cm^{-1}) of our QD suspensions to ionic strength (mol L^{-1}) we use the empirical relation:[6]

$$I = 12\kappa - 0.0002 \tag{S1}$$

This empirical relation can be verified in the following way. For an ideal solution (activity coefficients $\gamma_i = 1$) the ionic strength I (mol L^{-1}) is defined as:

$$I = \frac{1}{2}\sum_i z_i^2 c_i \tag{S2}$$

where z_i is the charge on the ion and c_i is the molar concentration. The conductivity κ (S cm^{-1}) of a solution is given by:

$$\kappa = F\sum_i |z_i| c_i u_i \tag{S3}$$

where u_i is the mobility (cm^2 V^{-1} s^{-1}). Combining equations (S2) and (S3):

$$I(\kappa) = \frac{\kappa}{2F} \sum_i \frac{|z_i|}{u_i} \tag{S4}$$

For a 1:1 electrolyte ($z_+ = 1$, $z_- = -1$) and taking $u_i = 8 \times 10^{-4}$ cm^2 V^{-1} s^{-1} (typical for ions such as K$^+$ and Cl$^-$), we obtain:

$$I = 13\kappa \tag{S5}$$

This equation, calculated for a 1:1 electrolyte is in reasonable agreement with eq. (S1). The Figure below shows a comparison of the values of ionic strength obtained from the measured conductivities for unthiolated SA/MHPC QDs using eq. (S1) and eq. (S5).
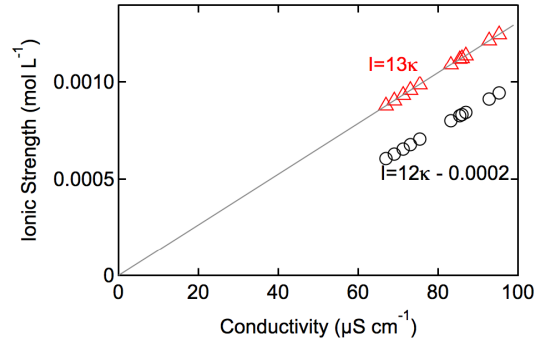
Figure S2. Ionic strength obtained from the measured conductivities for unthiolated SA/MHPC QDs using eq. (S1) and eq. (S5).

The zeta potential is given by:

$$\zeta = \frac{q}{4\pi\varepsilon\varepsilon_0 r} - \frac{q}{4\pi\varepsilon\varepsilon_0\left(r + \lambda^{-1}\right)} \tag{S6}$$

where r is the particle radius (7.0 x 10^{-9} m), q is the charge on the particle (1.602 x 10^{-19} C), ε_0 is permittivity of free space (8.854 x 10^{-14} F cm^{-1}), ε is the relative permittivity (80). The fits to the experimentally measured zeta potential using eqs. (S1) and (S5) are indistinguishable, as shown in Figure S2.
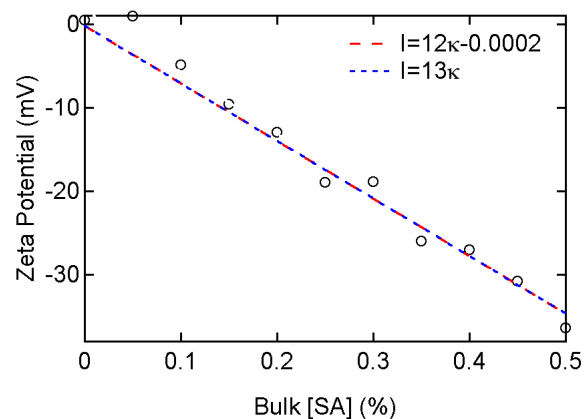
Figure S3. Zeta potential versus bulk [SA] mol% on unthiolated QDs. (o) Experimentally measured zeta potentials. The dashed lines are fits to the experimental data.

Correlation between zeta potential and fraction recovered

There is a strong correlation between the magnitude of the zeta potential and the fraction of QDs recovered after water solubilization.
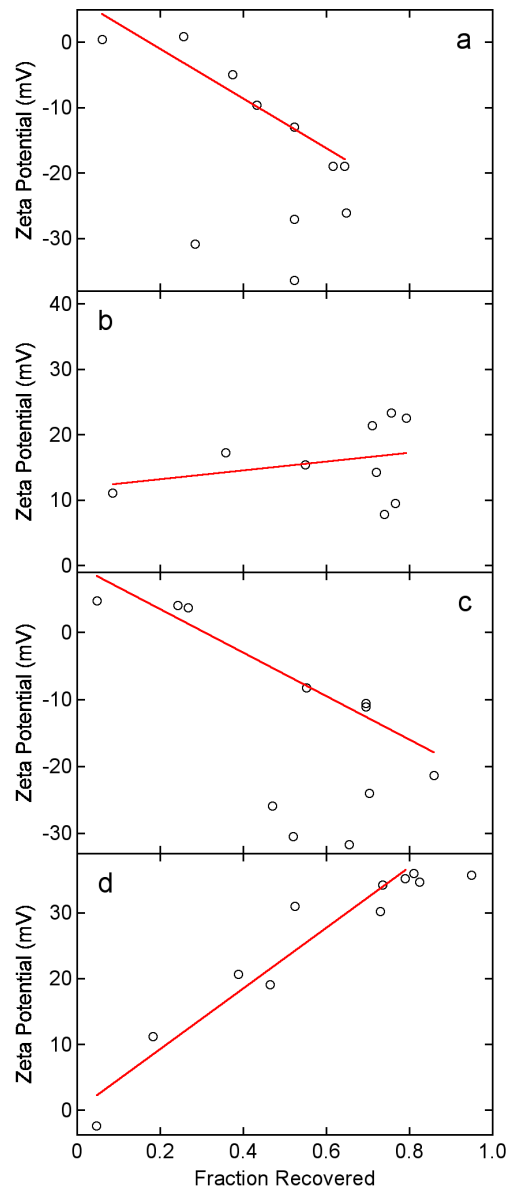
Figure S4. Zeta potential versus fraction recovered. The solid line represents a linear regression from 0 mol% to 30 mol% for (a) SA/MHPC on unthiolated QDs ($r^2 = 0.89$), (b) ODA/MHPC on unthiolated QDs ($r^2 = 0.11$), (c) SA/MHPC on thiolated QDs ($r^2 = 0.93$), (d) ODA/MHPC on thiolated QDs ($r^2 = 0.90$).

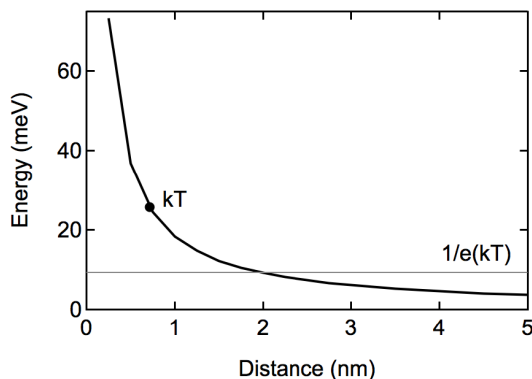**Electrostatic repulsion between point charges**

Figure S5. Interaction energy versus distance for two point charges taking $\varepsilon = 80$.

The plateau in the fraction of charged lipids, inferred from the zeta potential, is likely due to electrostatic repulsion between charged ODA groups. The interaction energy E(r) between two point charges at separation r is given by:

$$E(r) = \frac{q_1 q_2}{4\pi\varepsilon\varepsilon_0 r} \tag{4}$$

SA/MHPC or ODA/MHPC outer leaflet and dodecanethiol inner leaflet

SA/MHPC outer leaflet and dodecanethiol inner leaflet (Fig. S6a)

The absorbance spectra before and after water solubilization with 20 mol% SA and MHPC (Fig. S6b) show a significant increase in the second exciton peak at 500 nm that can be directly attributed to the thiol inner leaflet and consistent with the stronger interaction with the QD shell in comparison to HDA/TOPO.[7]

The addition of SA to the outer leaflet progressively increases the fraction of QDs recovered after water solubilization up to a maximum of 85% recovered (Fig. S6c). The size distributions for the thiolated QDs with 20 mol% and 30 mol% SA (Fig. S6d) reveal average sizes of 13.6 ± 0.7 nm and 14.7 ± 0.6, respectively, in good agreement with the values obtained for a SA/MHPC outer leaflet on unthiolated QDs. These results indicate that the replacing HDA/TOPO with a 1-dodecanethiol inner leaflet does not influence the average particle size.

The zeta potential for the thiolated QDs (Fig. S6e) decreased monotonically with increasing bulk mole fraction of SA. From the fit, we determine that 4.1% of the SA molecules in the surface layer are charged, similar to the value of 4.6 % obtained for unthiolated QDs with SA/MHPC. This indicates that thiolation does not significantly influence the driving force for formation of the outer leaflet. The long-term stability of the thiolated QDs with 25 mol% SA (Fig. S6f), as measured by the fraction recovered, remains unchanged for at least 3 months, just as with unthiolated QDs (Fig. 2f).
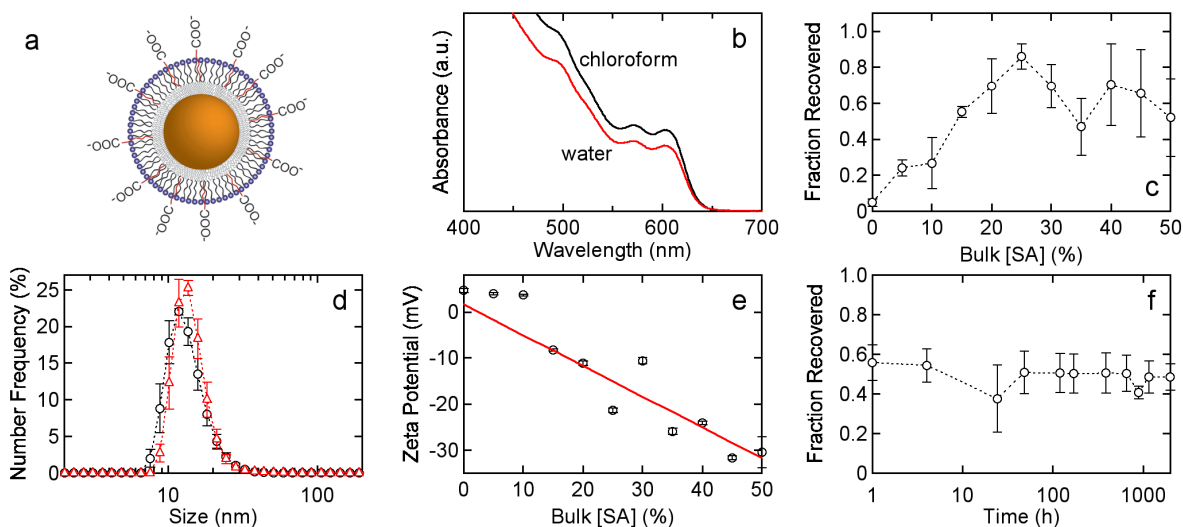

Figure S6. (a) QD lipid functionalization scheme: 1-dodecanethiol inner leaflet and a SA/MHPC outer leaflet. (b) Absorbance spectrum for QDs with 20 mol% SA before and after water solubilization. (c) Fraction of QDs recovered versus SA concentration 30 minutes after water solubilization. (d) Size distributions for QDs with (o) 20 mol% and (Δ) 30 mol% SA with averages sizes 13.6 ± 0.7 nm and 14.3 ± 0.6 nm, respectively. (e) Zeta potential versus mole fraction of SA. The solid line represents a least squares fit that corresponds to 4.1% charged (deprotonated) SA groups. (f) The stability of QDs functionalized with 25 mol% SA versus time.

SA/MHPC outer leaflet and dodecanethiol inner leaflet (Fig. S7a)

The absorbance spectra for thiolated QDs with 20 mol% ODA before and after water solubilization (Fig. S7b) show the same features as the thiolated QDs functionalized with SA/MHPC. The fraction of QDs recovered after water solubilization and filtration increases with ODA concentration, reaching a maximum of about 80% recovered at 25 mol% ODA. Size

distributions for 20 and 30 mol% ODA (Figure S7d) show single peaks at 13.6 ± 1.9 nm and 13.7 ± 0.8 nm, respectively, indicating monodispersed QDs. The size distributions are similar to unthiolated QDs indicating the thiolation does not affect formation of the outer leaflet.

The zeta potential increases linearly with bulk ODA concentration (Figure S7e) up to a value of about 30 mV at 30 mol% ODA. From the fit, we determine that the concentration of charged ODA molecules in the lipid layer is 9.1%, somewhat larger than the value of 5.2 % obtained for unthiolated QDs (Fig. S7e). The well-defined plateau suggests that the surface concentration saturates at 2.7 mol% charged ODA corresponding to 24 charged ODA molecules per QD.

The long-term stability of the thiolated QDs with 25 mol% ODA remains very high, greater than 90%, for at least 3 months (Fig. S7f) and is consistent with both SA/MHPC and ODA/MHPC functionalized QDs.
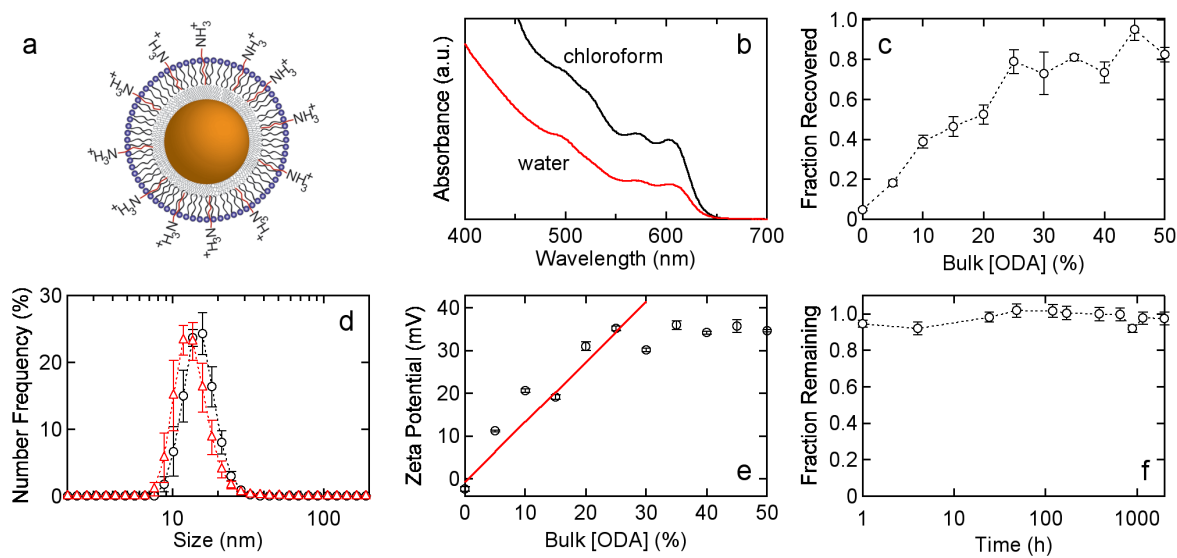

Figure S7. (a) QD lipid functionalization scheme: 1-dodecanethiol inner leaflet and an ODA/MHPC outer leaflet. (b) Absorbance spectra for QDs with 20 mol% ODA before and after water solubilization. (c) Fraction of QDs recovered versus ODA concentration 30 min after water solubilization. (d) Size distributions for QDs with (o) 20 mol% and (Δ) 30 mol% ODA with averages sizes of 13.6 ± 1.9 nm and 13.7 ± 0.8 nm, respectively. (e) Zeta potential versus mole fraction of ODA. The solid line represents a least squares fit that corresponds to 9.1% charged (protonated) ODA groups. (f) The stability of QDs functionalized with 25 mol% ODA versus time.

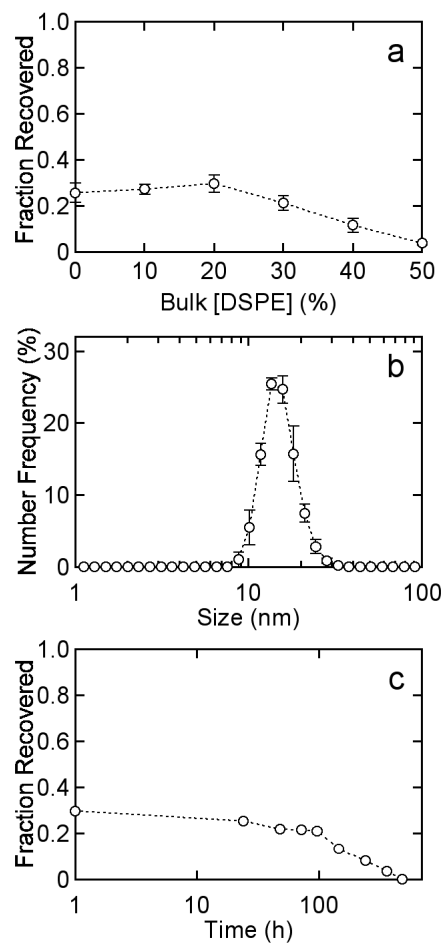**DSPE/MHPC outer leaflet and TOPO/HDA inner leaflet**

Figure S8. (a) Fraction of QDs recovered versus the mole fraction of DSPE 30 min after water solubilization. The QDs were functionalized with DSPE and MHPC. (b) Size distributions for 20 mol% DPSE. The average size was 16.7 ± 2.1 nm. (c) Fraction of QDs recovered versus time for QDs functionalized with 20 mol% DSPE. A drop in stability is observed after approximately 100 h.
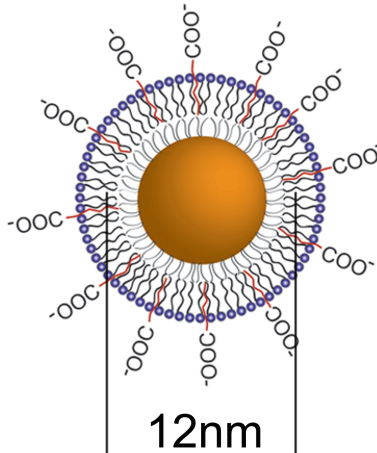
Determination of number of Lipids per QD

Figure S9. Schematic illustration of a lipid coated QD.

The number of lipids in the outer leaflet was estimated from the surface area of the particle and the area per molecule. Due to the high curvature, the surface area was calculated based on the diameter at the mid-point of the outer leaflet (12 nm). The area per molecule was taken as 0.25 nm^2 for SA[3] and 0.64 nm^2 for MHPC.[2] A rule of mixtures was used to determine the number of lipids:

$$\frac{\text{Lipids}}{\text{QD}} = \frac{A_{QD}}{(0.3 \cdot A_{SA} + 0.7 \cdot A_{MHPC})} \qquad (S7)$$

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