Supporting information for

7-Mercapto-4-methylcoumarin as a reporter of thiol binding to the CdSe quantum dots surface

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Pages S2-S3: Experimental details and synthesis of core CdSe OD558 and (Cm-S-).

Pages S4-S5: Figure S1: ¹H-RMN of a) Cm-SH, b) core-shell QD485 c) TOPO and Cm-SH d) a mixture of QD485 and Cm-SH in CDCl₃.

Page S6: Figure S2: UV-Visible spectra of core-shell QD485 (2.54 μ M), (Cm-S-)₂ (2.9 μ M) and the mixture of both in toluene.

Page S6: Figure S3: UV-Visible spectra at ratios [Cm-SH]/[QD] up to 30 in toluene.

Page S7: Figure S4: Changes in $(Cm-S)_n-QD485$ fluorescence intensity as a function of sample aging.

Page S7: Figure S5: Excitation and emission spectra of the $(Cm-S)_n-QD485$ supramolecular structures.

Page S8: Figure S6: UV-Visible spectra of Cm-SH in basic or acid methanol.

Page S8: Figure S7: Fluorescence spectra of core-shell (A) QD485 or (B) QD550 upon incremental addition of Cm-SH (up to 15 μ M) in toluene (λ_{exc} = 420 nm).

Page S8: Table S1. QD and $(Cm-S)_n$ -QD lifetimes in ns.

Page S9: Figure S8: Fluorescence decay traces of core-shell QD550 upon additions of Cm-SH up to 15 μM in deaerated toluene. A) λ_{exc} = 440 nm, λ_{em} > 550 nm and B) λ_{exc} = 395 nm, λ_{em} < 500 nm.

Page S9: Figure S9: UV-Visible and emission spectra of the synthesized core CdSe QD558.

Page S10: Figure S10: UV-Visible of core CdSe QD558 in absence and in presence of increasing amounts of Cm-SH.

Page S10: Figure S11: Emission spectra of core CdSe QD558 in absence and in presence of increasing amounts of Cm-SH at two different excitation wavelengths (373 or 450 nm).

Page S11: Figure S12: Stern-Volmer plots for the quenching of core-shell QD485 and core CdSe QD558 by Cm-SH.

Page S12: Figure S13: Fluorescence decay traces of core CdSe OD558 upon additions of Cm-SH up to 15 µM in deaerated toluene. A) λ_{exc} = 440 nm, λ_{em} > 550 nm and B) λ_{exc} = 395 nm, λ_{em} < 500 nm.

Experimental Details:

Cm-SH was purchased from Fluka and recrystallized prior to use. CdSe/ZnS QD were purchased from Evident Technologies and the experiments were carried out in toluene solution at room temperature without QD purification (i.e., we did not eliminate any excess of TOPO free in solution) since our objective was not quantify the amount of TOPO that can be released upon capping exchange with Cm-SH. All the fluorescence measurements were recorded under nitrogen (we have noticed that the emission of the new assembly, $(Cm-S)_n$ -QD, is air sensitive) with a Photon Technology International (PTI) spectrofluorometer. Fluorescence lifetime measurements were recorded using an Easylife LS (PTI) using the appropriate pulsed LED diodes and filters for each case.

¹H NMR spectra were obtained on a Bruker Avance-300 spectrometer at 300 MHz using $CDCl₃$ as solvent with tetramethylsilane as an internal standard.

Details of UV-Visible or steady state fluorescence quenching experiments:

In a typical experiment, 2 mL of a toluene solution of QD 2.54 μ M or 0.51 μ M (for UV-Visible or fluorescence, respectively) was prepared in a precision quartz cuvette For fluorescence studies the sample was deaerated at least 20 min with N_2 .

Added to the cuvette were varying amounts of a 0.19 mM Cm-SH stock solution in toluene such that the total volume added to each cuvette was $200 \mu L$ or less.

Synthesis of core CdSe QD558:

It is well-known that the ZnS shell protects QD from quenching.¹ In order to learn more about the shell effect in our system, we have synthesized core CdSe QD protected by a layer of trioctylphosphine oxide (TOPO) ligands with emission at 558 nm using the procedure reported by Peng and Peng.² Briefly, Se (56.6 mg) was dissolved in trioctylphosphine (2.5 mL) to make a TOPSe solution. Cadmium oxide (53.0 mg), tetradecylphosphonic acid (223.7 mg), and TOPO (3.78 g) were heated to 300 $^{\circ}$ C under a N₂ flow in a 25mL three-necked round bottom flask equipped with a stir bar and until the solution turned clear and colorless.

This mixture was allowed to cool to 270°C, and the TOPSe solution was injected into the reaction flask under strong stirring. The reaction was stopped 2h 30 min after the injection by transferring the reaction mixture from the flask into ice-cold methanol. After washing with methanol and centrifugation at 4000 rpm during 20 min $(3x)$, the OD were redispersed in toluene and kept in the dark.

We have observed these new assemblies $(Cm-S)_n-QD$ by UV-Vis and steady-state fluorescence by using the same procedure as above. However, upon excitation at 373 nm, as the new band appears, the QD fluorescence was being quenched (in contrast with the core-shell QD550), which confirms that the ZnS shell is protecting the QD from quenching (See ESI7-10).

Synthesis of $(Cm-S-)_{2}:^{2}$

Using a mortar and pestle, 7-mercapto-4-methylcoumarin (151.3 mg, 0.7871 mmol), KMnO4 $(127.0 \text{ mg}, 0.8036 \text{ mmol})$ and neutral Al_2O_3 (88.8 mg, 0.871 mmol) were grinded together for 10 min. The product was extracted using a generous amount of dichloromethane and the resulting mother liquor was evaporated to dryness using a rotatory evaporator. The residual solid was recrystallized from ethanol and dichloromethane to afford 41.4 mg (28% yield) of pale yellow needles. ¹H NMR (CDCl₃, 400 MHz): δ 7.51 (*J*=8.2 Hz, d, 1H, *Ar*H), 7.41 (*J*=2 Hz, d, 1H, *Ar*H), 7.37 (*J*1=8.2 Hz, *J*2=1.8 Hz, q, 1H, *Ar*H), 6.23 (*J*=0.8 Hz, d, 1H, *=C*H), 2.39 (*J*=0.8 Hz, d, 3H, *C*H3). 13C NMR (CDCl3, 400 MHz): δ 160.14, 153.97, 151.80, 140.81, 125.25, 122.08, 119.05, 114.93, 114.36, 18.63. m.p. 234-245°C. MS (EI) m/z 382.03 (24.4), 318.10 (20.1), 290.05 (32.8), 191.01 (55.5), 163.02 (49.9), 91.1 (100.0), 77.04 (50.9), 68.98 (65.6) . HRMS Calcd. for C₂₀H₁₄O₄S₂: 382.0334. Found: 382.0314.

Fig. S1. b) ¹H-RMN of core-shell QD485 in CDCl₃.

Fig. S1. d) ¹H-RMN of a mixture of core-shell QD485 and Cm-SH in CDCl₃.

Effect of $(Cm-S-)$ on QD485 absorbance:

Fig. S2. UV-Visible spectra of core-shell QD485 (blue line, 2.54 μ M), (Cm-S-)₂ (green line, $2.9 \mu M$) and the mixture of both in toluene (black line).

Fig. S3: UV-Visible spectra of 0.51 µM QD485 with incremental addition of Cm-SH up to 15 µM in toluene (ratio [Cm-SH]/[QD485] up to 30).

Fig. S4: Changes in $(Cm-S)_n-QD485$ fluorescence intensity as a function of sample aging $(\lambda_{\text{exc}} = 373, \lambda_{\text{mon}} = 434)$.

Fig. S5. Normalized excitation (λ_{exc} 240-430 nm, λ_{em} 440 nm, solid line) and b) emission (λ_{exc} = 373 nm, dashed line) spectra of the $(\text{Cm-S})_n$ -QD485 supramolecular structures. The emission spectrum shown is the difference between QD485 with and without Cm-SH.

Fig. S6. UV-Visible spectra of Cm-SH under acidic (0.5M H₂SO₄, blue, labelled "+Acid") or basic (0.22 triethylamine. Orange, labelled "+Base) conditions in methanol. The blue dashed line ($\lambda_{\rm exc}$ =330 nm) corresponds to Cm-SH emission under acid conditions, the orange dashed line ($\lambda_{\rm exc}$ =380 nm) and green solid line ($\lambda_{\rm exc}$ =308 nm) to Cm-SH emission under basic conditions. Fluorescence spectra are normalized on the right axis. Under basic conditions the species present in solution is the anionic form.³

Fig. S7: Fluorescence spectra of core-shell (A) QD485 or (B) QD550 (both 0.51 µM, under N₂) upon incremental addition of Cm-SH (up to 15 μ M) using λ_{exc} = 420 nm in toluene.

Fig. S8: Fluorescence decay traces of core-shell QD550 (0.51 µM) upon incremental addition of Cm-SH up to 15 μ M in deaerated toluene. A) λ_{exc} = 440 nm, λ_{em} > 550 nm and B) $\lambda_{\rm exc}$ = 395 nm, $\lambda_{\rm em}$ < 500 nm. The IRF signal has been added in both graphs for comparison (shorter decay for panel A, dashed line for panel B).

Fig. S9: Normalized UV-Visible (black dashed line) and emission (orange solid line) spectra of core CdSe QD558 (no ZnS shell) in toluene.

Peng et al.⁴ found the relationship between the first exciton peak and the nanoparticle size. By using this equation the size and extinction coefficient per mole of our CdSe QD were calculated (552 nm absorption peak) and found to be 3.1 nm and 1.1×10^5 M⁻¹cm⁻¹, respectively.

Fig. S10: UV-Visible of core CdSe QD558 (2.54 µM) upon addition of Cm-SH up to 18.5 µM in toluene.

Fig. S11: Emission spectra of core CdSe QD558 (0.51 µM) upon increasing additions of Cm-SH up to 20 μ M in deaerated toluene at two different excitation wavelengths A) 373 nm and B) 450 nm.

Fig. S12: Stern-Volmer plots for the quenching of core-shell QD485 (black solid circles, $\lambda_{\rm exc}$ = 465 nm, $\lambda_{\rm em}$ = 480 nm) and core CdSe QD558 (black circles outlines, $\lambda_{\rm exc}$ = 450 nm, $\lambda_{\rm em}$ $= 558$ nm) by Cm-SH.

In order to obtain the quantitative characteristics of the fluorescence quenching process, Stern-Volmer experiments were carried out. When aliquots of the Cm-SH solutions are added to the QD solution, the decrease in the emission intensity is described by eq. 1,

$$
I_0/I-1=K_{SV}[Q](1)
$$

where K_{SV} is the Stern-Volmer constant. Thus, we obtained the following values:

 K_{SV} =7.9x10⁴ M⁻¹ (Stern-Volmer constant for core-shell QD485 quenching by Cm-SH). K_{SV} =2.9x10⁴ M⁻¹ (Stern-Volmer constant for core CdSe QD558 quenching by Cm-SH).

Fig. S13: Fluorescence decay traces of core CdSe QD558 (0.51 µM) upon incremental addition of Cm-SH up to 15 μ M in deaerated toluene. A) λ_{exc} = 440 nm, λ_{em} > 550 nm and B) λ_{exc} = 395 nm, λ_{em} < 500 nm. The IRF signal has been added in both graphs for comparison (shorter decay for panel A, dashed line for panel B).

References:

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