

Electronic Supplementary Information For:

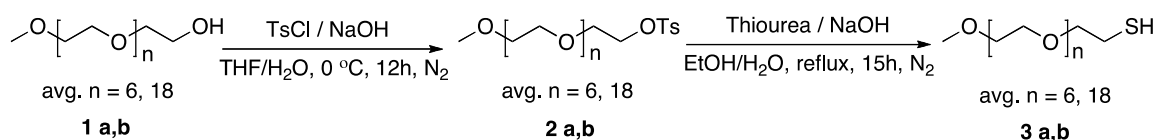
Enhancing the Physicochemical and Photophysical Properties of Small (<2.0 nm) CdSe Nanoclusters for Intracellular Imaging Applications

Katie N. Lawrence,[§] Sukanta Dolai,[§] Yu-Hung Lin,[§] Amala Dass,[#] and Rajesh Sardar^{§,*}

[§]Department of Chemistry and Chemical Biology, Indiana University Purdue University Indianapolis, Indianapolis, IN 46202, United States. [#]Department of Chemistry and Biochemistry, University of Mississippi, Oxford, MS 38677, United States.

*E-mail: rsardar@iupui.edu

Synthesis of PEG-thiols. Different chain length PEG_n-thiols (n = 6 and 18) were synthesized according to the literature procedure⁽¹⁾ with modification.



1. Synthesis of Poly(ethylene glycol)-methyl ether-*p*-toluene sulfonate (MeO-PEG₆-OTs) 2a.

In a 250 mL two-neck round bottom flask, 20 g (67.48 mmol) of Poly(ethylene glycol)-monomethyl ether (MeO-PEG₆-OH, M_n = 350, **1a**) was dissolved in 25 mL THF and stirred for 20 min under ice-cold condition. In another flask, 3.42 g (85.5 mmol) of NaOH was dissolved in 25 mL distilled water. The NaOH solution was slowly added to **1a** under ice-cold condition. Separately, *p*-Toluene sulfonyl chloride (13 g, 68.18 mmol) was dissolved in 50 mL THF and added drop-wise via a dropping funnel to the reaction mixture at ice-cold condition for a time period of 30 min under N₂ atmosphere. The temperature of the reaction mixture was maintained between 0-10 °C for an additional 30 min and then left to warm up slowly to room temperature while stirring. After stirring at room temperature for 12 h, the crude reaction mixture was transferred to a separatory funnel and washed with EtOAc (3 X 100 mL). Organic layers were collected and washed with distilled water and brine solution, and then dried over anhydrous

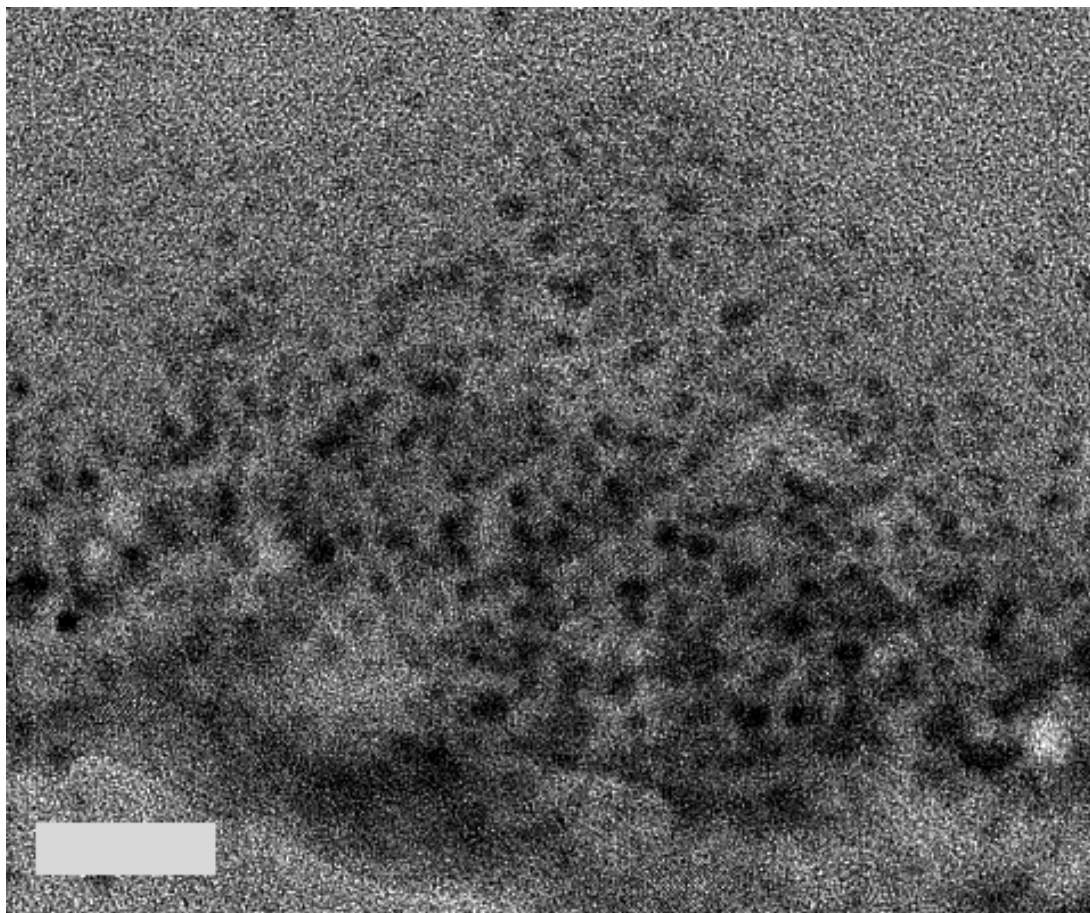
Na₂SO₄. Solvent was then removed by rotatory evaporator to yield the product **2a** as colorless viscous liquid. Yield: ~24 g (79 %). ¹H NMR (500 MHz, CDCl₃) δ ppm: 2.43 (3H, s); 3.36 (3H, s); 3.52-3.68 (22H, m); 4.13-4.15 (2H, t); 7.32-7.34 (2H, d); 7.77-7.79 (2H, d). ¹³C NMR (125 MHz, CDCl₃) δ ppm: 21.62, 59.0, 68.65, 69.25, 70.50, 70.55, 70.59, 70.72, 71.92, 127.96, 129.81, 133.01, 144.77. ESI-MS (m/z): 336 (n=3), 380.2 (n=4), 424.3 (n=5), 468.3 (n=6), 512.3 (n=7), 556.3 (n=8), 597.3 (n=9), 641.3 (n=10), 685.5 (n=11); where n = number of -CH₂-CH₂-O- repeat unit. Under identical molar ratios of reagents and exactly similar reaction conditions, **2b** was synthesized from **1b**.

2. Synthesis of Poly(ethylene glycol)-methyl ether-thiol (MeO-PEG₆-SH) 3a. In a 250 mL two-neck round bottom flask, 10 g (22.2 mmol) of MeO-PEG₆-OTs and 2.5 g (33.3 mmol) thiourea were dissolved in 100 mL anhydrous EtOH and the reaction mixture was allowed to reflux under N₂ atmosphere for 12 h. Next, 1.4 g (35 mmol) of NaOH was dissolved in 20 mL distilled water and added to the reaction mixture. The reaction mixture was then cycled with vacuum and argon for three times and refluxed for 3h. After cooling down the reaction mixture to room temperature, ethanol was evaporated using a rotary evaporator then diluted with 100 mL of distilled water, and acidified with 0.5 (M) HCl to pH ~3. The product was collected using DCM (3 X 50 mL), which was again washed with water, dried over anhydrous Na₂SO₄, and solvent was evaporated to yield the MeO-PEG₆-thiol (**3a**) as colorless viscous liquid. Yield: 7.95 g (86%). ¹H NMR (500 MHz, CDCl₃) δ ppm: 1.16-1.58 (1H, t); 2.64-2.68 (2H, m); 3.34 (3H, s); 3.48-3.65 (22H, m). ¹³C NMR (125 MHz, CDCl₃) δ ppm: 24.24, 58.99, 70.2, 70.48, 70.50, 70.55, 70.60, 71.90, 72.84. ESI-MS (m/z): 267.1 (n=3), 311.3 (n=4), 355.2 (n=5), 399.3 (n=6), 443.3 (n=7), 487.2 (n=8), 531.3 (n=9), 575.3 (n=10); where n = number of -CH₂-CH₂-O- repeat unit. Under identical molar ratios of reagents and exactly similar reaction conditions, **3b** was synthesized from **2b**.

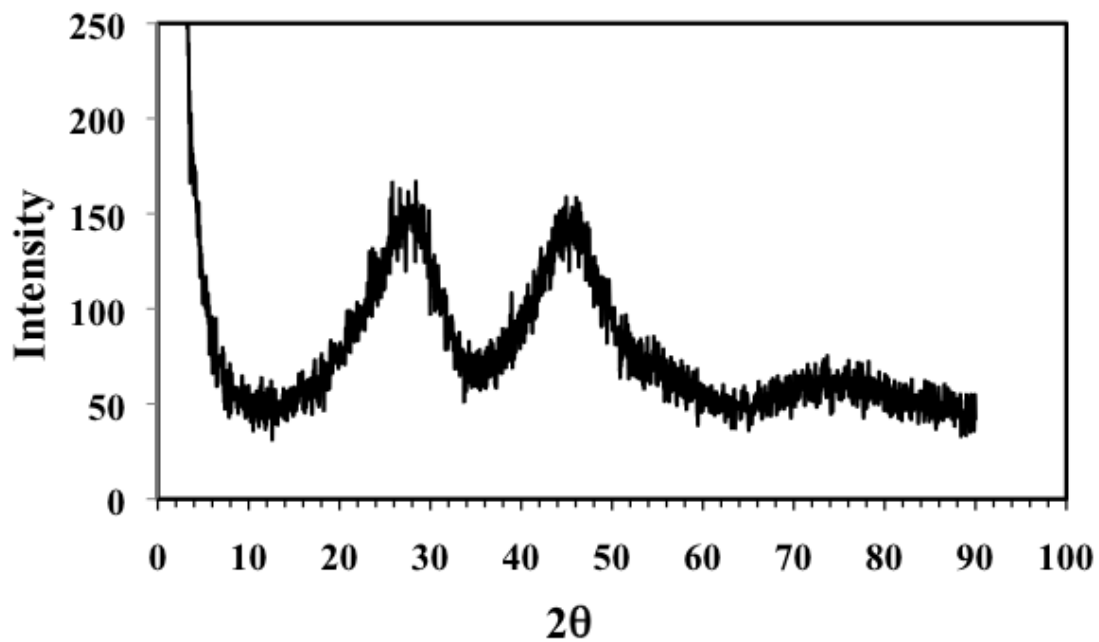
NMR Analysis: The ¹H NMR spectrums were collected with a Bruker AVANCE III 500 instrument at 500MHz working frequency. Approximately 10 mg of samples were dissolved in 0.6mL of DCM-d at room temperature and a 500 scans were collected with a 30° pulse angle with 3.17 sec of acquisition and 3 sec of relaxation time. ³¹P NMR was also collected using the same instrument working at 220 MHz. All acquired data were plotted via TOPSPIN 2.0 software.

X-ray Photoelectron Spectroscopic (XPS) Analysis: X-ray Photoelectron Spectroscopic (XPS) analysis were performed on a Kratos Axis Ultra DLD system with an Mg anode at 1253.6 eV and X-ray power of 150 W. A charge neutralizer was used to prevent charging. The survey scans were collected from binding energies of 0-1000 eV with a 80 eV pass energy. All data were collected so that the C 1s line was shifted to 284.6 eV. The measurements were conducted at a pressure <5x10⁻⁹ Torr. For XPS analysis, the samples were drop-casted on a piranha-cleaned silicon wafer inside a N₂ filled glove box and the solution was allowed to evaporate at room temperature. The piranha-cleaned silicon wafer was washed with copious amount of Nanopure water and ethanol, and then dried under high vacuum overnight. (Warning: piranha solution is very corrosive and must be handled with extreme caution. It reacts violently with organic materials and may not be stored in tightly closed vessels).

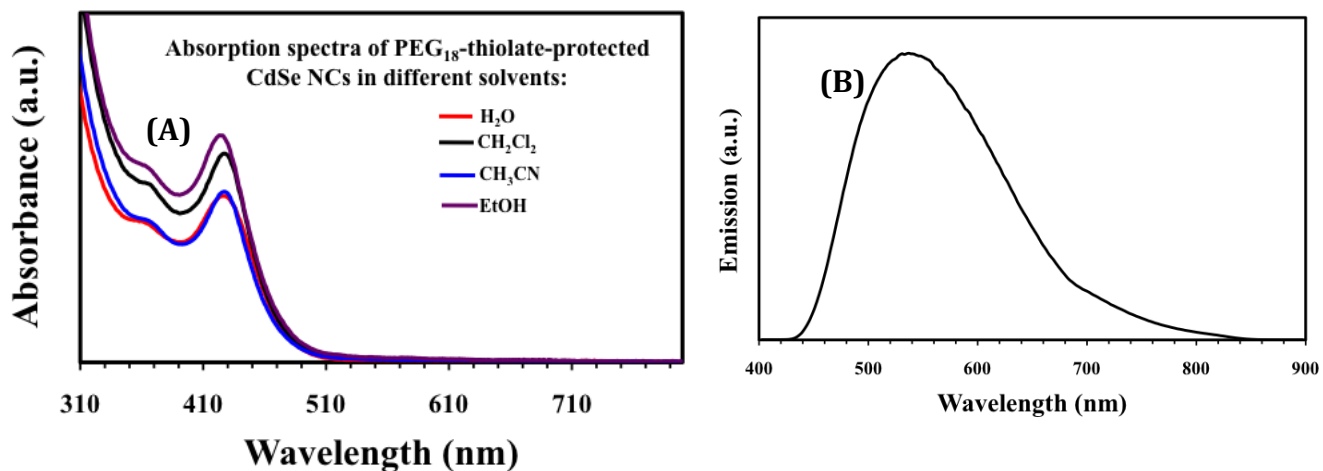
List of Figures



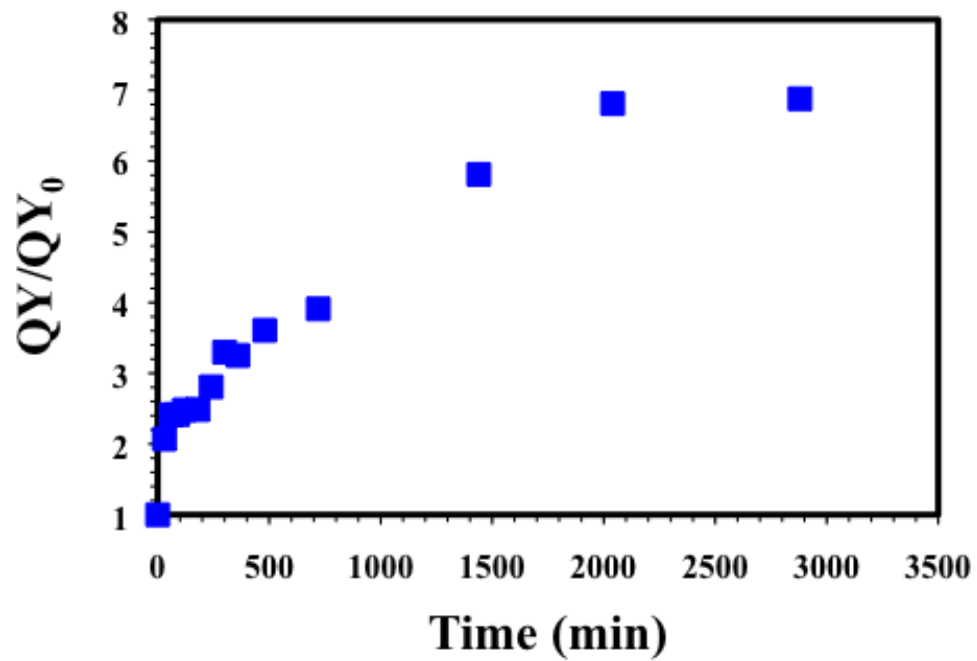
ESI-Fig. 1 TEM image of PEG₆-thiolate-protected CdSe NCs with lowest energy absorption peak at 429 nm. The NCs are 1.7 nm in diameter. Scale bar is 10 nm.



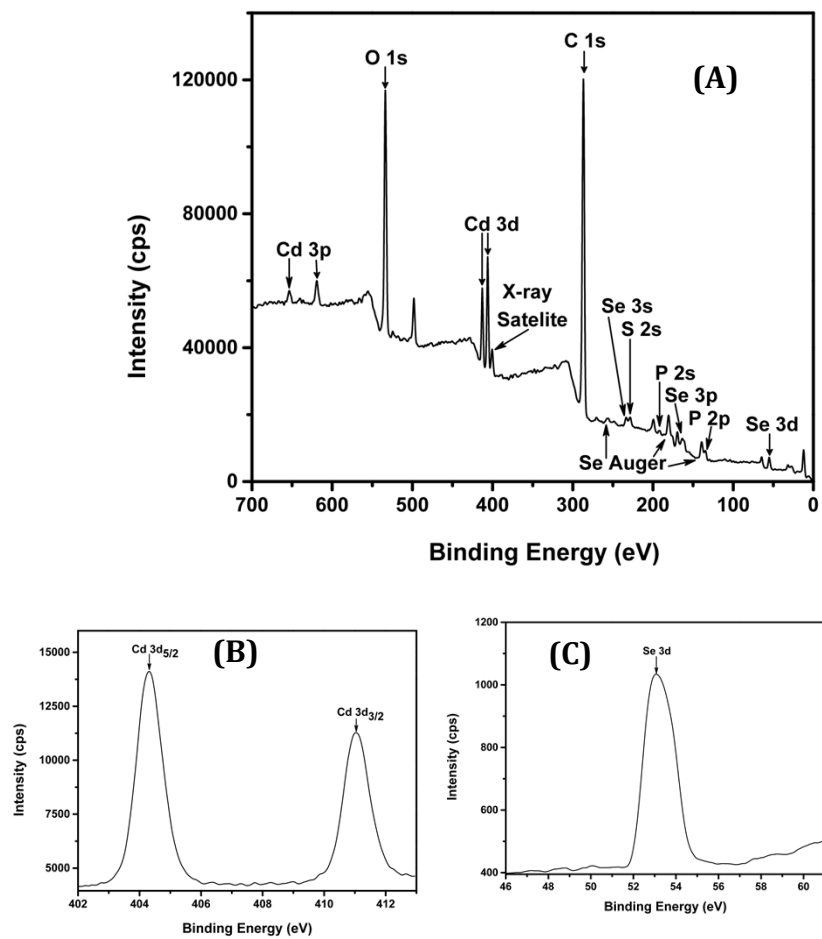
ESI-Fig. 2 Powder XRD of purified PEG₆-thiolate-protected CdSe NCs.



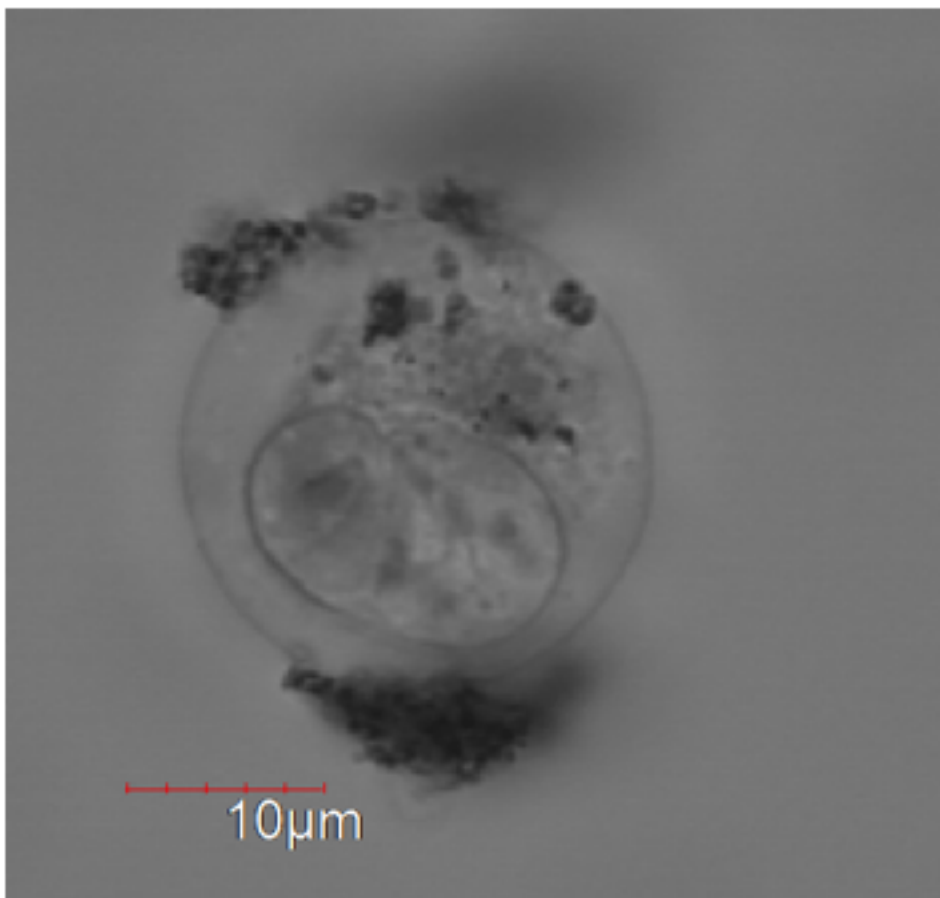
ESI-Fig. 3 (A) Absorption spectra of purified PEG₁₈-thiolate-protected CdSe NCs in different solvent. (B) PL spectrum of purified PEG₁₈-thiolate-protected CdSe NCs in CH₂Cl₂, and (C) TEM images of PEG₁₈-thiolate-protected CdSe NCs after phase transfer and purification. Scale bar is 10 nm. Few single isolated NCs were in 1.6 nm diameters. The larger NCs observed could be due to the aggregation process during the TEM sample preparation.



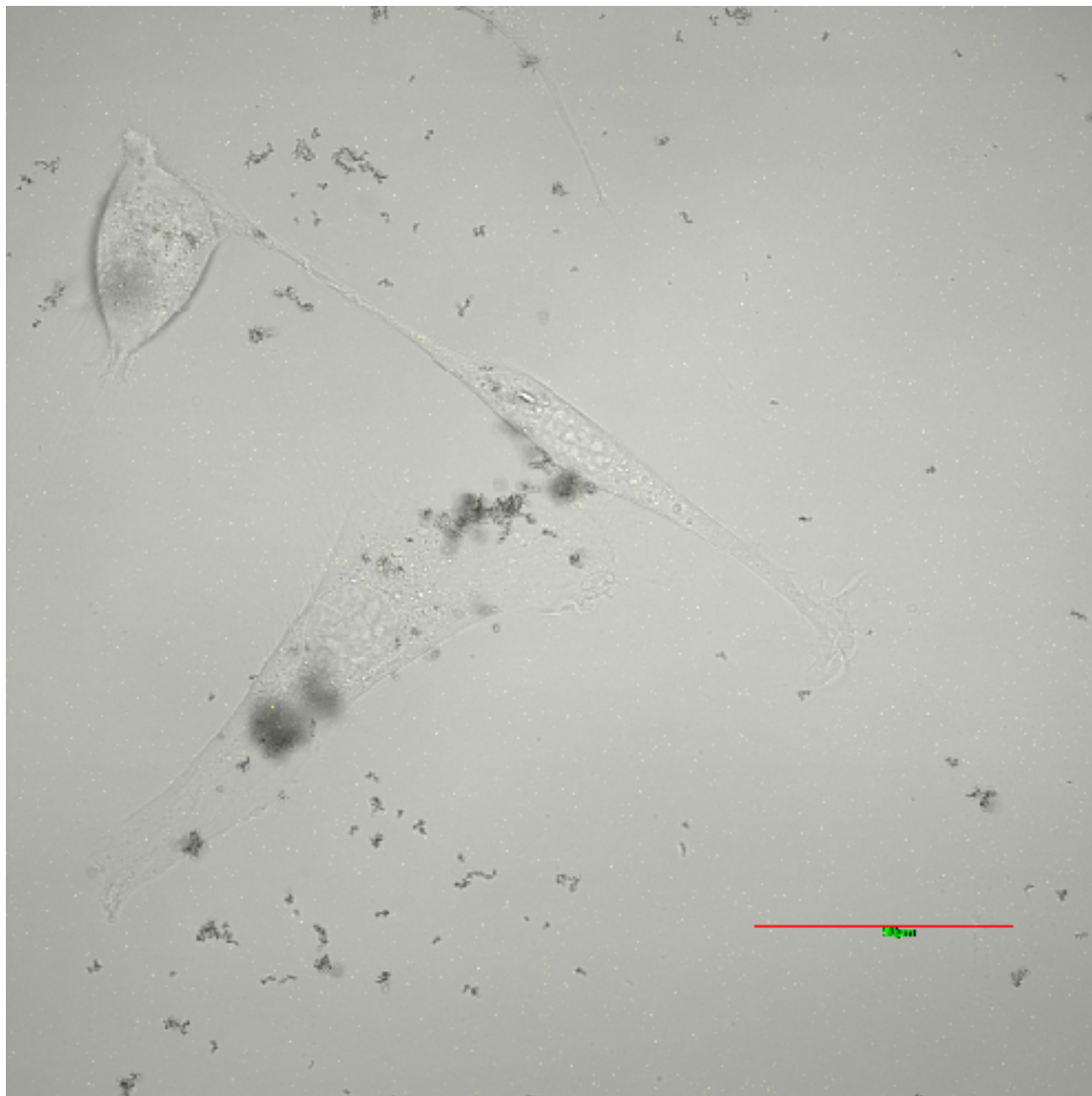
ESI-Fig. 4 Normalized PL QY of PEG₁₈-thiolate-protected ultra-small CdSe NCs in DCM after addition of TPP.



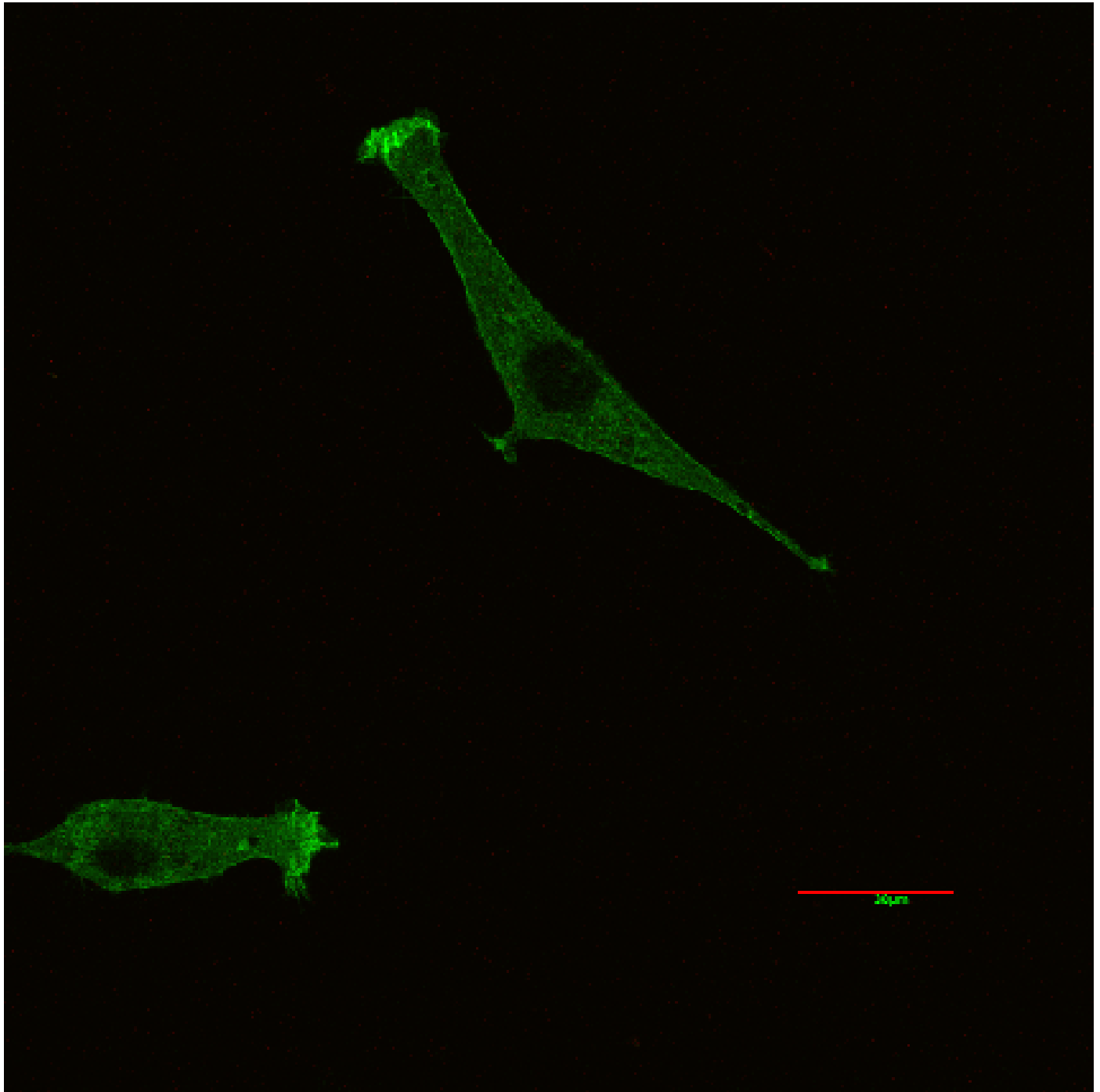
ESI-Fig. 5 (A) XPS survey scan of TPP-treated PEG₆-thiolate-protected CdSe NCs. High resolution scan of (B) Cd 3d region, (C) Se 3d region.



ESI-Fig. 6 DIC image of fibroblast cells incubated with ~ 100 nM TPP-treated PEG₆-thiolate-protected CdSe NCs. Most of the NCs are aggregated around the outer cell membrane. The NCs, which are present inside the cell, do not display any fluorescence properties.

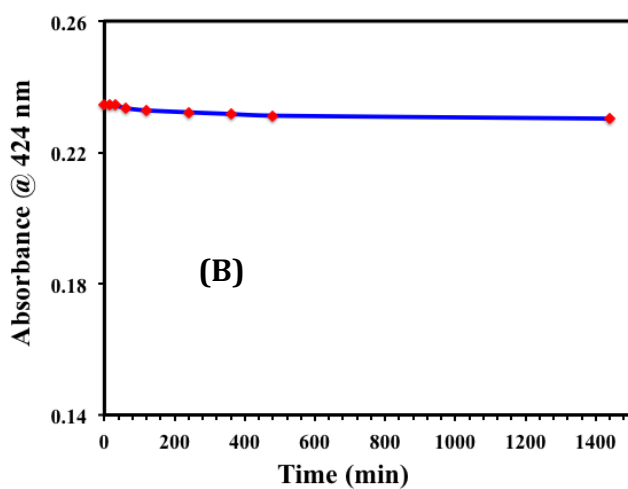
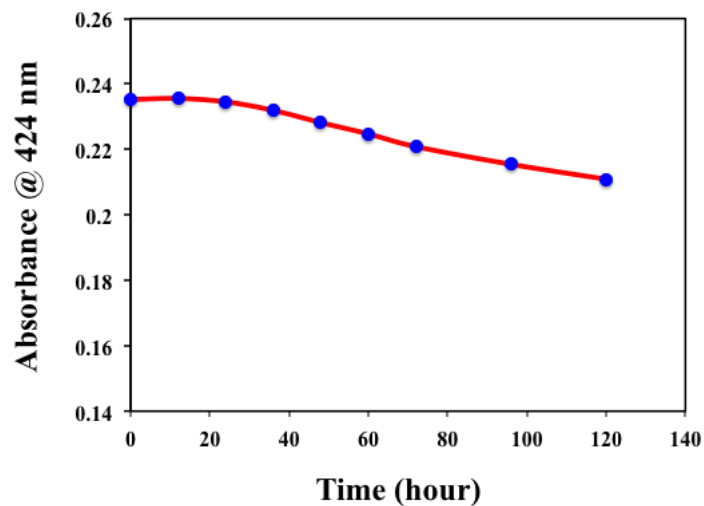


ESI-Fig. 7 DIC image of fibroblast cells incubated with ~100 nM TPP-treated PEG₁₈-thiolate-protected CdSe NCs. Because of the ultrasmall size of CdSe NCs it was difficult to visualize uptake NCs inside the cells.



ESI-Fig. 8 Representative fluorescence image of merged NCs and green fluorescence protein transfected cells.

(A)



ESI-Fig. 9 (A) Stability of mixed TPP- and PEG₁₈-thiolate-coated CdSe NCs for a period of 5 days upon dissolved in cell culture media and left under dark. (B) Stability of mixed TPP- and PEG₁₈-thiolate-coated CdSe NCs for a period of 24 hrs upon dissolved in nanopure water and exposed to 365 nm UV light for 60 seconds.

ESI-Table 1. Comparison of hydrodynamic radii, polydispersity index, and Zeta-potential for two different PEG-thiolate coated CdSe NCs.

| CdSe NC coating | Hydrodynamic radii (nm) | Polydispersity index | Zeta-potential (mV) |
|-----------------------------|-------------------------|----------------------|---------------------|
| PEG ₆ -thiolate | 4.25 ± 6.4 | 0.354 | -19.2 ± 2.17 |
| PEG ₁₈ -thiolate | 4.50 ± 4.4 | 0.217 | -32.0 ± 2.08 |

Reference:

1. Snow, A. W.; Foos, E. E. Conversion of Alcohols to Thiols via Tosylate Intermediates. *Synthesis* **2003**, *4*, 509-512.