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Cross-Linked, Glassy Styrenic Surfactants Stabilize Quantum Dots Against Environmental Extremes

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Supplemental Material

Materials. Spectrophotometric grade *N*,*N*'-dimethylformamide (DMF) and tetrahydrofuran (THF) were purchased from Aldrich and used as received. Water was purified with a Milli-Q water system (18 MΩ). TOPO-capped quantum dots (QDs) used in encapsulation were purchased from Evident Technologies (all EviDots; CdSe@ZnS: "Maple-Red-Orange", catalog #ED-C11-TOL-0620; CdTe@ZnS: "Empire Red", catalog #EM-C11-TOL-0680; CdSe: "Begonia Red", catalog #EL-C11-TOL-0610). Water-solubilized quantum dots used in stability comparisons were purchased from Evident (EviTag Type 1, catalog #ET-C11-CB1-0620; and Evitag Type 2, catalog #E2-C11-CB2-0620) and Quantum Dot Corporation (Qdot605 ITK, COOH-functionalized, catalog #2130-1). Poly(styrene-*block*-acrylic acid) (PS_{250}-*b*-PAA_{13}; M_n = 27,000 g/mol) was prepared as previously described.[1] All other reagents and solvents were used as received.

Characterization. UV-vis spectra were obtained on a Hewlett-Packard series 8453 UV-vis spectrophotometer equipped with an Agilent 89090A Peltier temperature. Fluorescence spectra were acquired with a Quantamaster Fluorimeter (PTI, London, Ontario) at room temperature, using emission and excitation slit widths of 2 μm (equivalent to λ = ±2 nm spectral width). For stability

tests (Figure 2), [QD] = 2.8 nM. For the measurements shown in Figure 2A, QD solutions in Fluorovettes (Aline, Inc., Redondo Beach, CA) were incubated in a 65 °C bath, but were cooled to 25 °C in a second bath before fluorescence intensity measurement in the Quantamaster fluorimeter. In these experiments excitation wavelength (λ_{ex}) was set to 400 nm, and emission wavelength (λ_{em}) to $\lambda_{em,max}$ of the nanoparticle. For experiments shown in Figure 2B, measurements were performed with a SpectraMax M2 plate reader (Molecular Devices, Sunnyvale, CA) using λ_{ex} = 365 nm and λ_{em} = $\lambda_{em,max}$ for each particle type.

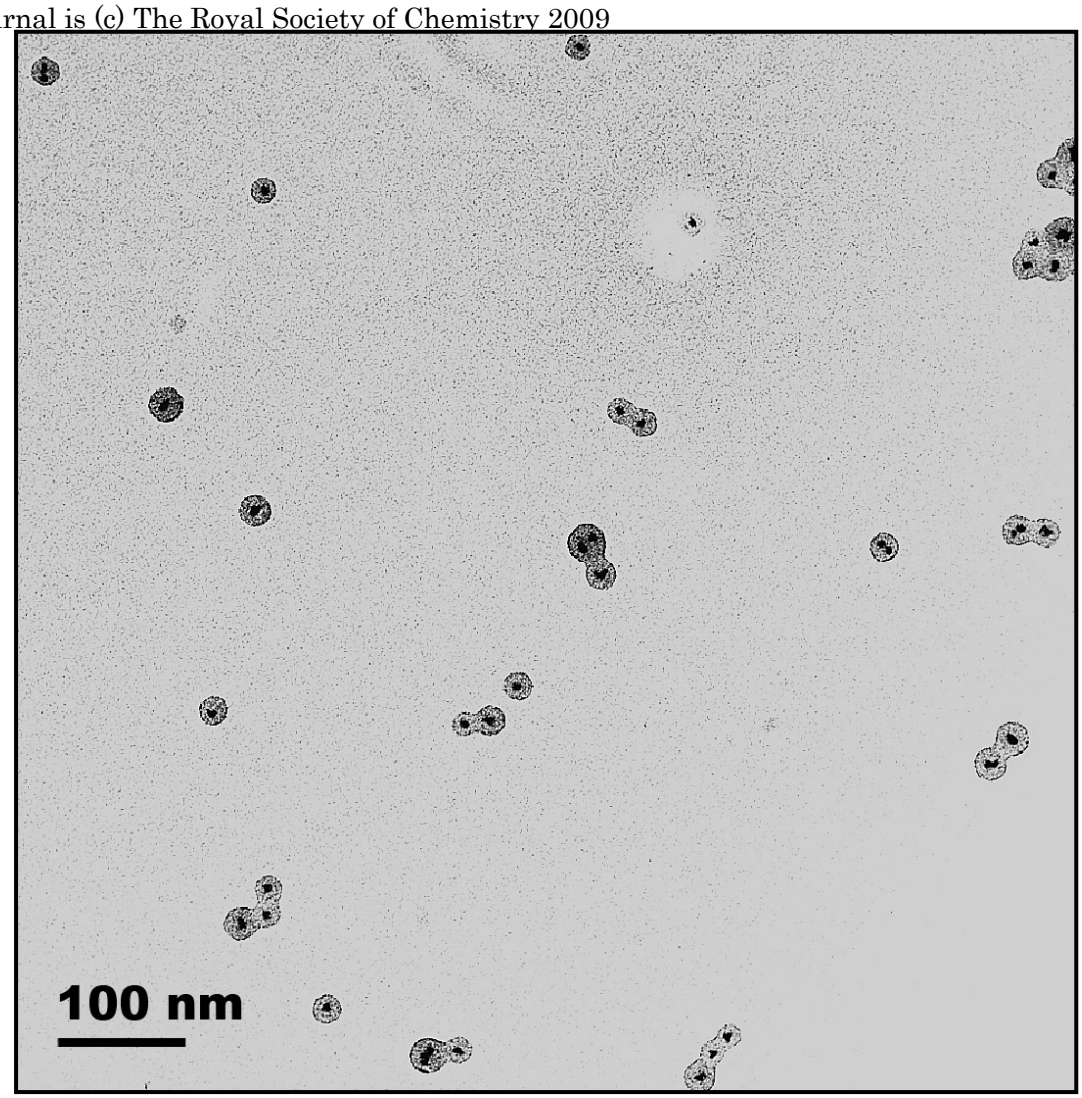
The critical water content (CWC) for micelle formation in solution, either in the presence or absence of QDs, was measured by configuring the Quantamaster to measure elastic light scattering (λ_{ex} = λ_{em}, ϕ = 90). Block copolymer was dissolved in DMF and/or THF to 1 mg/mL, and the scattered light intensity was collected with each addition of 100 μL water. Quantum yields were determined by the comparison of spectrally integrated emission of QD-micelles in water with the emission of Rhodamine 6G dissolved to identical optical density (< 0.015) at the same excitation wavelength (λ_{ex} = 400 nm). Transmission electron microscopy (TEM) images were obtained on a JEOL 1210 electron microscope equipped with a Gatan video camera and a Gatan Multiscan CCD camera (1024×1024 pixels). To prepare TEM samples, nanoparticle solution (5 μL) was diluted with 95 μL of H_2O, and 1.5 μL of the solution was dropped onto Formvar-graphite-coated copper grids (300 mesh, Electron Microscopy Science). The sample was air-dried for TEM imaging. All images were obtained at an operating voltage of 120 kV.

Encapsulation of QDs. All manipulations were performed under air at 20 ^{o}C unless otherwise mentioned. In a glass vial, 10 μL of PS_{250}-*b*-PAA_{13} in DMF or THF (10 mg/mL) was diluted with 190 μL of DMF and 400 μL of THF. Toluene was evaporated from EviDot TOPO-capped QDs, and then the dried QDs were redispersed in an identical volume of THF. This solution was added dropwise into the polymer solution with vigorous stirring, and the mixture was allowed to stir another 1.5 h. At this stage, the concentrations of the polymer $[PS_{250}\text{-}b\text{-}PAA_{13}]_{initial}$ and QDs $[QD]_{initial}$ were 1.67 x 10^{-4} mg/mL and 9.24 x 10^{-3} mg/mL, respectively. Into the mixed solution, 6 mL of H_2O was added with a syringe pump at a rate of 140 mL/h, to induce the formation of the micelles around the QD. The solution was stirred for 30 min, and was then subjected to dialysis (Spectra/Por® 4 Regenerated Cellulose Membrane, MWCO = 12-14K) against Millipore water for 12 h, twice, to remove organic solvents. This procedure yielded singly encapsulated particles. Beginning with a solution of polymer in THF alone, and adding water to the particle-polymer mixture at 400 mL/h, yielded micelles containing multiple particles.

Micelle shell cross-linking. The number of carboxylates available for cross-linking in the assembled micelle solution was first calculated based on the starting polymer concentration, and reagents were added based on this calculation. For example, to cross-link 25% of carboxylates in the above solution, 3.5 μL of freshly prepared (3-dimethylamino)propyl)-3-ethylcarbodiimide methiodide (EDC) solution (1.0 mg/mL in H_2O, 1.2 x 10^{-8} mol EDC) was added and stirred for 1 h. Then, 8.9 μL of 2,2'-(ethylenedioxy)bis(ethylamine) solution (0.10 mg/mL in H_2O, 6.0 x 10^{-9} mol) was added and stirred for 24 h. Residual organic reagents were then removed by dialysis of the

suspension in a dialysis membrane (Spectra/Por® 4 Regenerated Cellulose Membrane, MWCO = 12-14K) against H_2O for 12 h.

***In vivo* injection and imaging in zebrafish.** Wild-type zebrafish were maintained and raised according to standard procedures.[2] Embryos were collected and kept at 30.5 °C in egg water with .003% propylthiouracil (PTU) to inhibit pigment formation. Embryos were manually dechorionated and were staged by hours post fertilization.[3] At 55 or 75 h post-fertilization, the embryos were anesthetized using 0.4 mg/ml 3-amino benzoic acid ethyl ester (Sigma A-5040) in egg water. 45 or 90 nL (for 2-day- or 3-day-old fish) of 140 nM solutions of CdSe@ZnS@PS-*b*-$PAA_{25\%XL}$, CdSe@ZnS-S-PEG-COOH (EviTag Type 1) and CdSe@ZnS@PE-PEG-COOH (EviTag Type 2) was injected into the common cardinal vein of the embryos using glass micropipettes as previously described.[4] The injected embryos were transferred in egg water to microscope slides and imaged 5 min and 40 min after injection using a Zeiss Axioplan 2 and ApoTome. Injected embryos were then maintained and could be imaged for two more days before sacrificing.



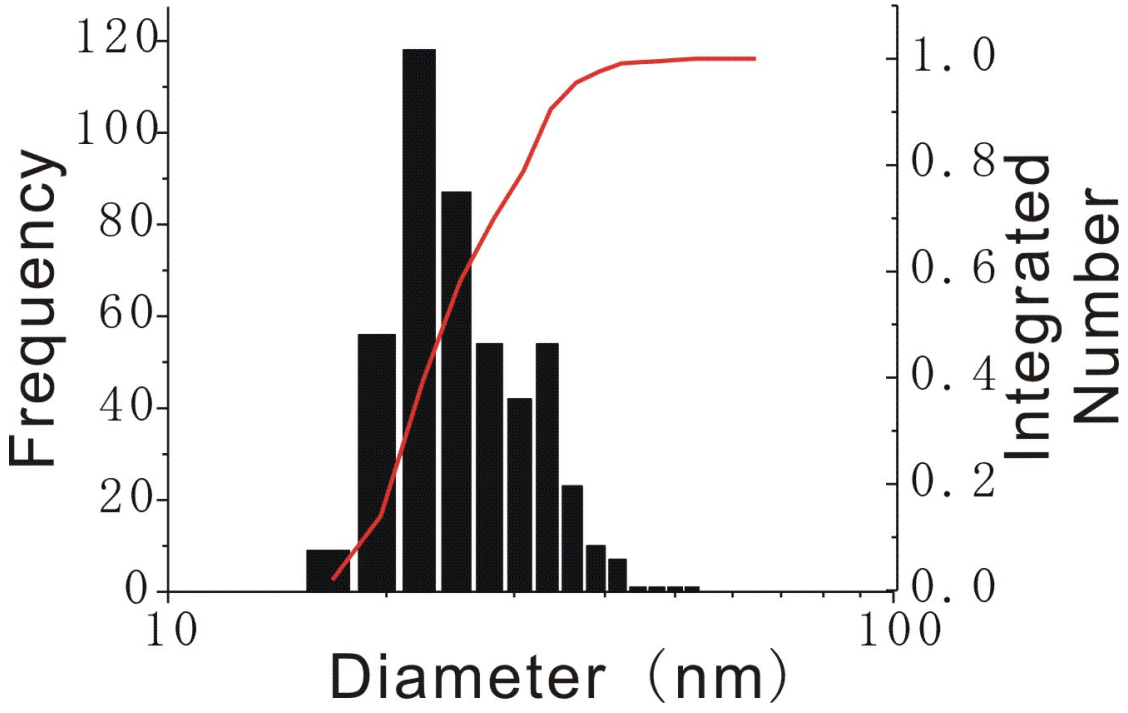


Figure S1. (Above) Low-magnification TEM image of CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$ obtained from a starting QD solution in 20:80 (*v*/*v*) DMF:THF. (Below) Histogram of total object diameter, built from this and 7 other TEM images.

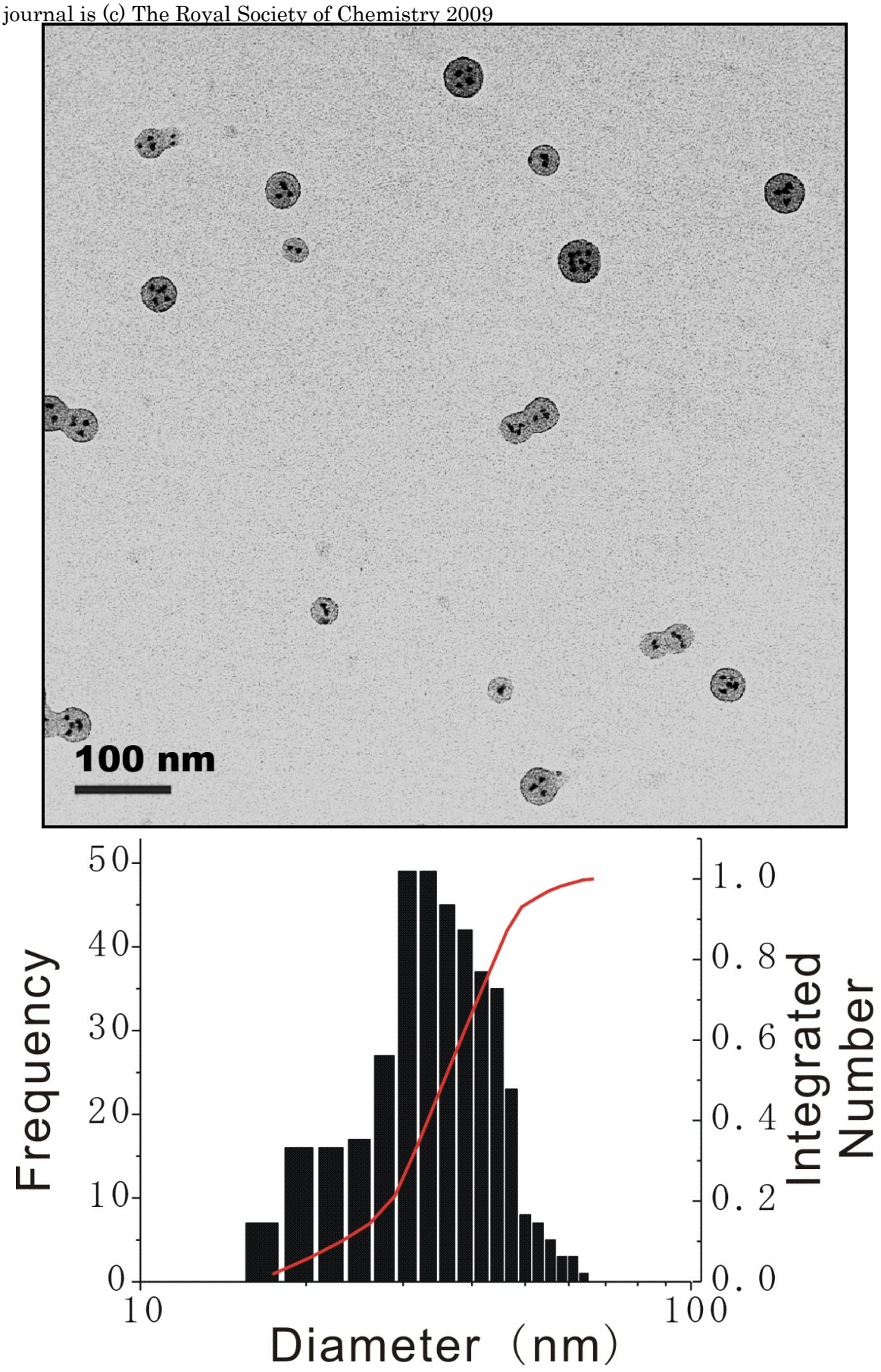


Figure S2. (Above) Low-magnification TEM image of CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$ obtained from a starting QD solution in 100% THF. (Below) Histogram of total object diameter, built from

this and 7 other TEM images.

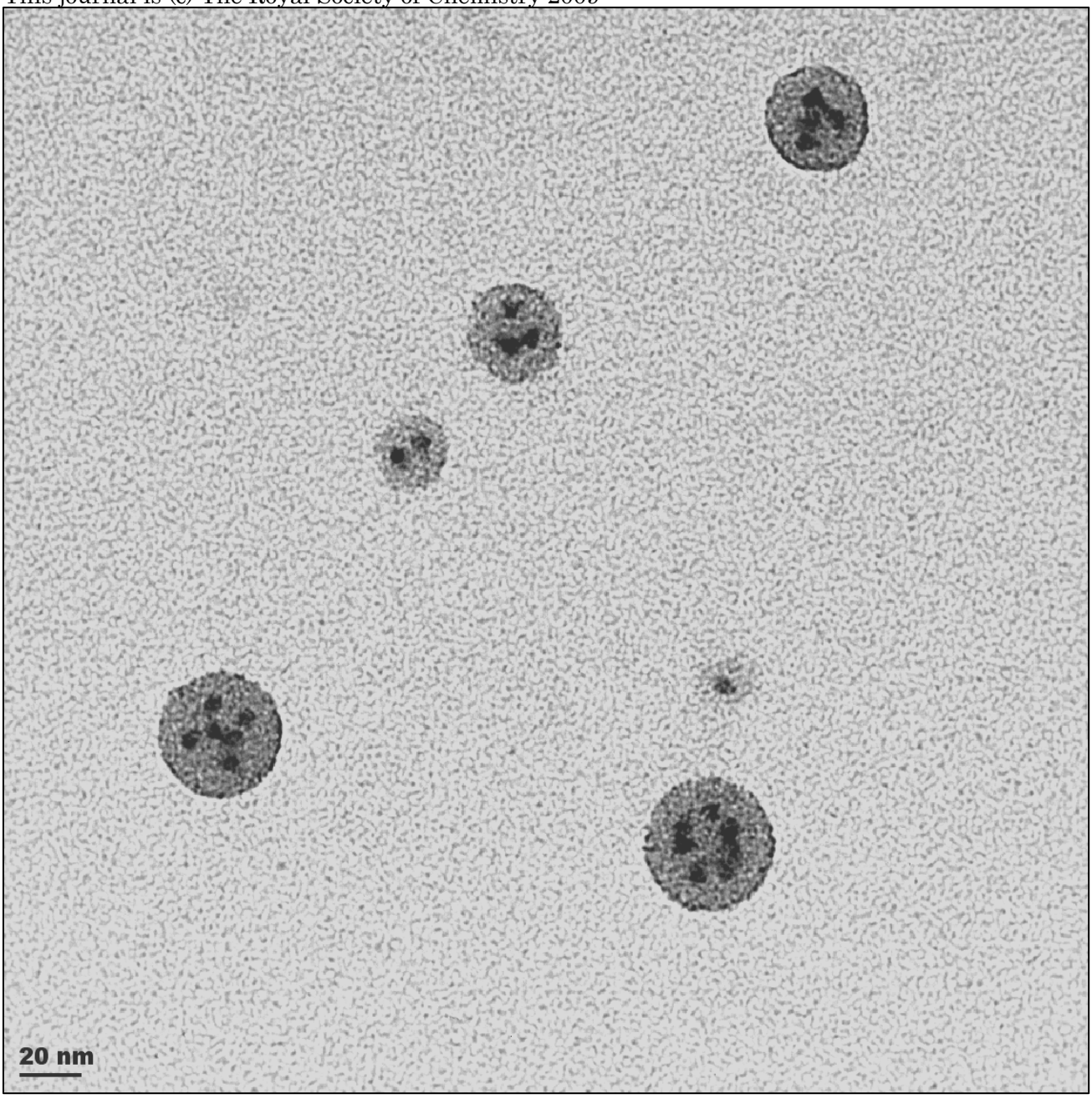


Figure S3. High-magnification TEM image of CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$ obtained from a starting QD solution in 100% THF.

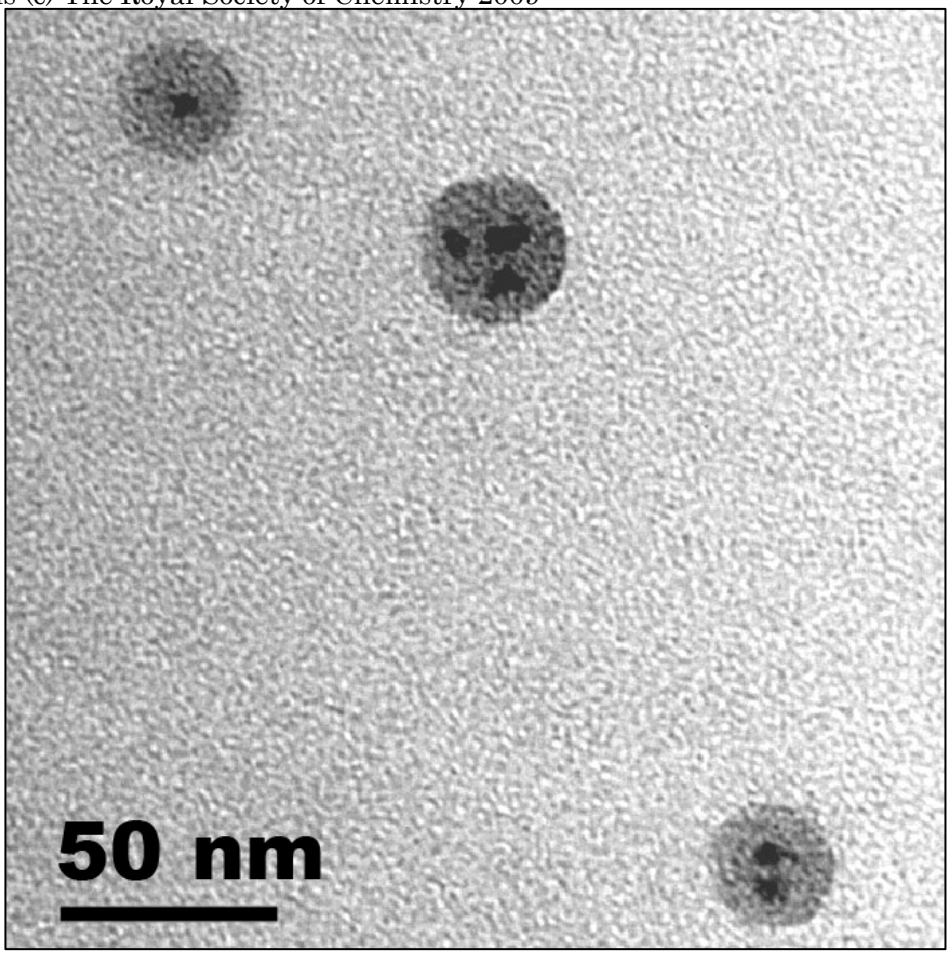


Figure S4. High-magnification TEM image of CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$ after 96 h in 2.5X TBE buffer. Solution was dialyzed against H_2O to remove salt before imaging.

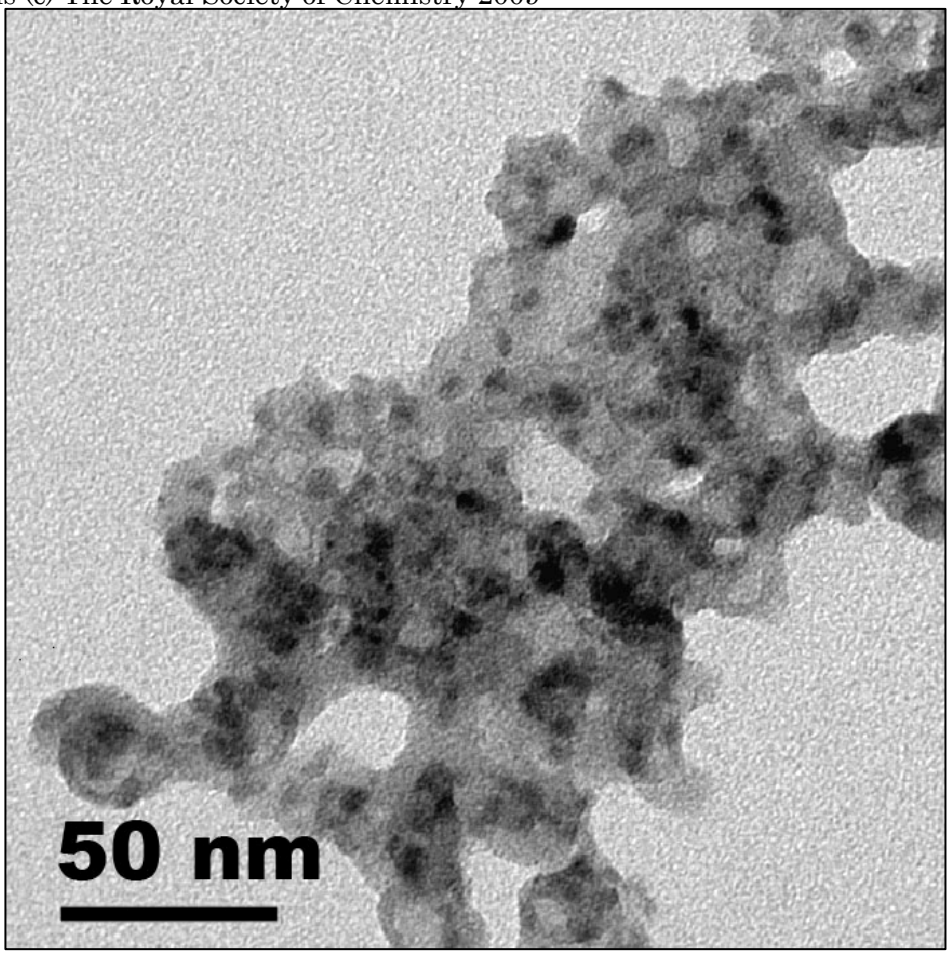


Figure S5. High-magnification TEM image of CdSe@ZnS@PE-PEG-COOH (EviTag Type 2) after 96 h in 2.5xTBE buffer. Solution was dialyzed against H_2O to remove salt before imaging.

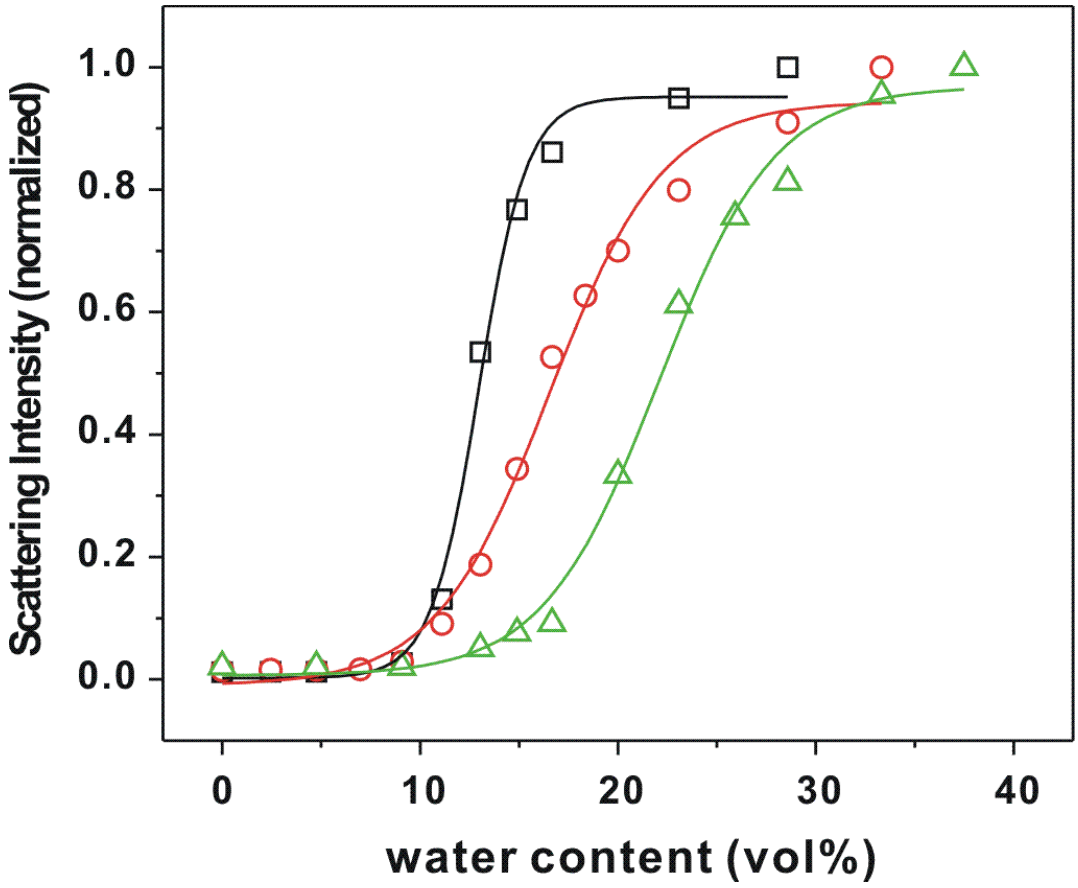


Figure S6. Static light scattering of PS_{250}-*b*-PAA_{13} solutions with added water. Beginning polymer solution is dissolved in ( ) 50:50 (*v*/*v*) DMF:THF, (O) 20:80 (*v*/*v*) DMF:THF, and (Δ) 100% THF. Respective critical water contents (CWCs) are χ_{H_2O} = 0.13, 0.16, and 0.23.

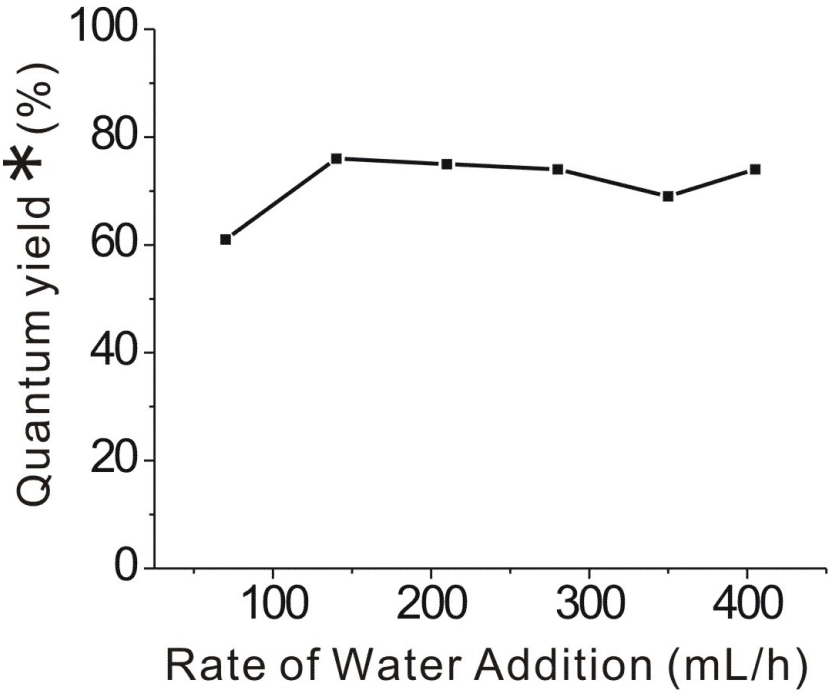


Figure S7. Dependence of relative quantum yield (QY*, calculated from the QY of the starting QD in toluene) of CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$ on the rate of water addition during the formation of the micelle encapsulated QDs.

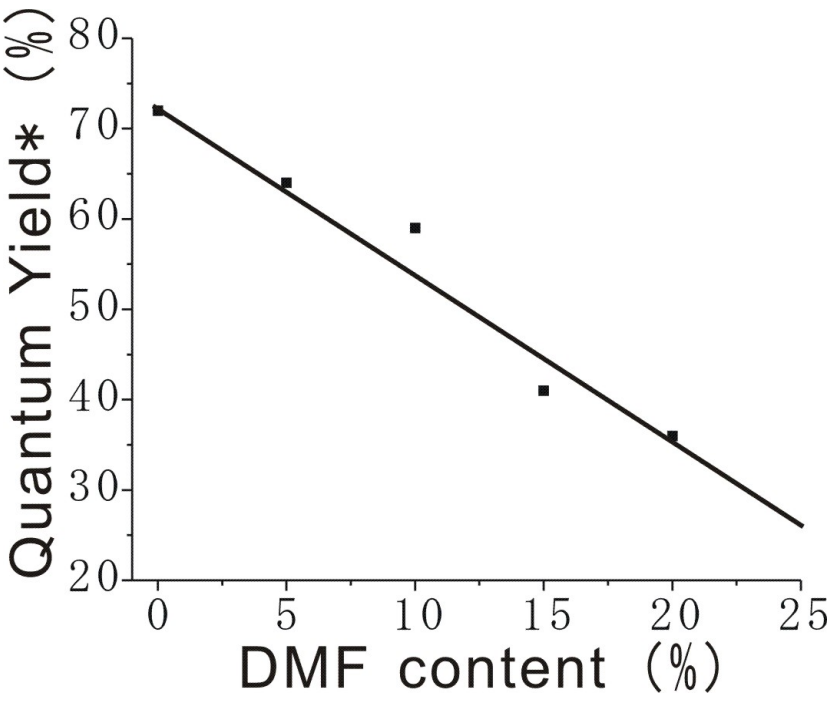


Figure S8. Dependence of relative quantum yield (QY*) of CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$ on the initial fraction of DMF (relative to THF) used in assembly of the copolymer shell.

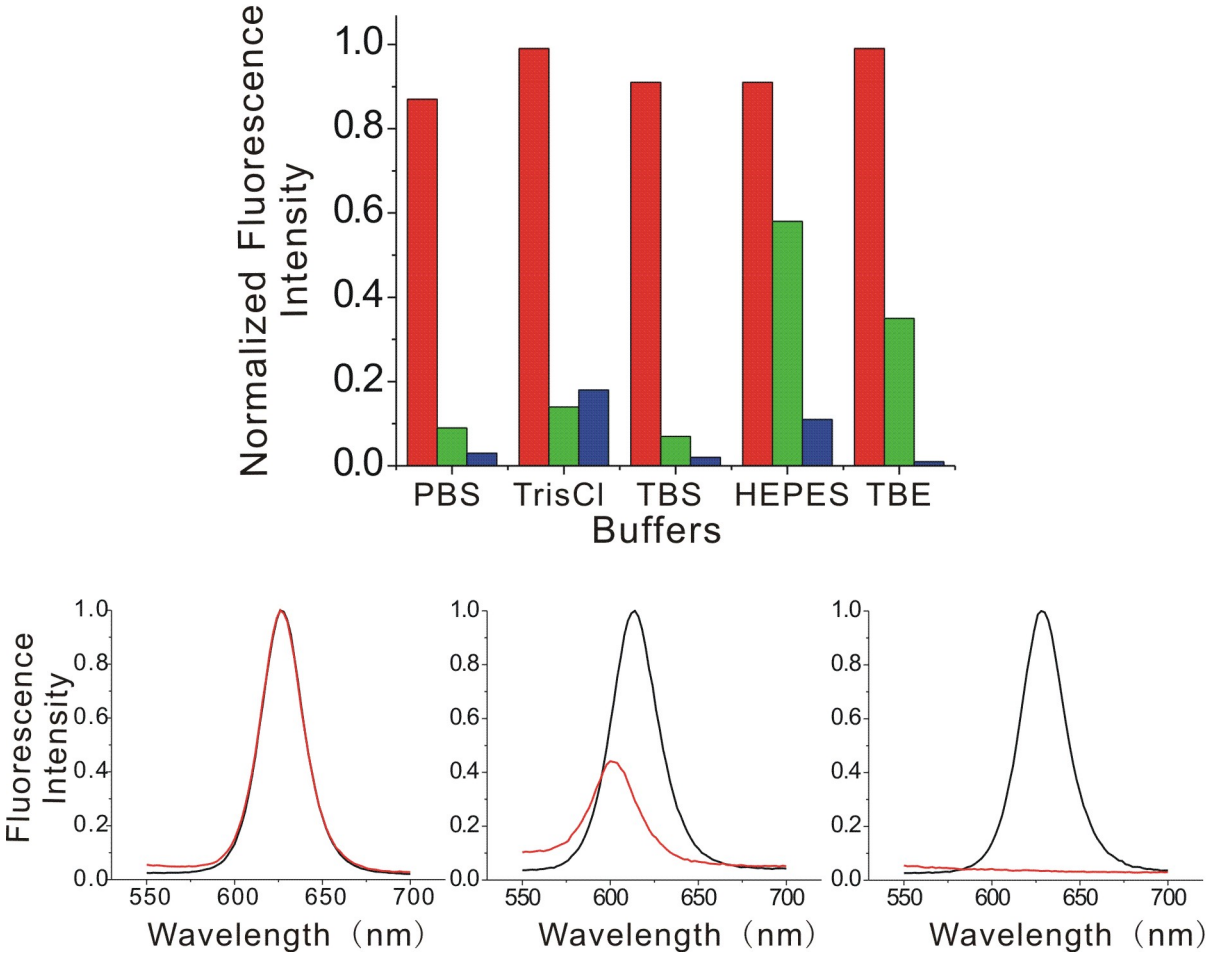


Figure S9. (Above) Stability of CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$ (red), CdSe@ZnS-S-PEG-COOH (EviTag Type 1, green), and CdSe@ZnS@PE-PEG-COOH (EviTag Type 2, blue) in various 2.5X buffers after 96 h. (Below) Fluorescence spectra of CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$ (left), CdSe@ZnS-S-PEG-COOH (center), and CdSe@ZnS@PE-PEG-COOH (right) before exposure to 2.5X TBE (black curve) and after 96 h in 2.5X TBE (red curve).

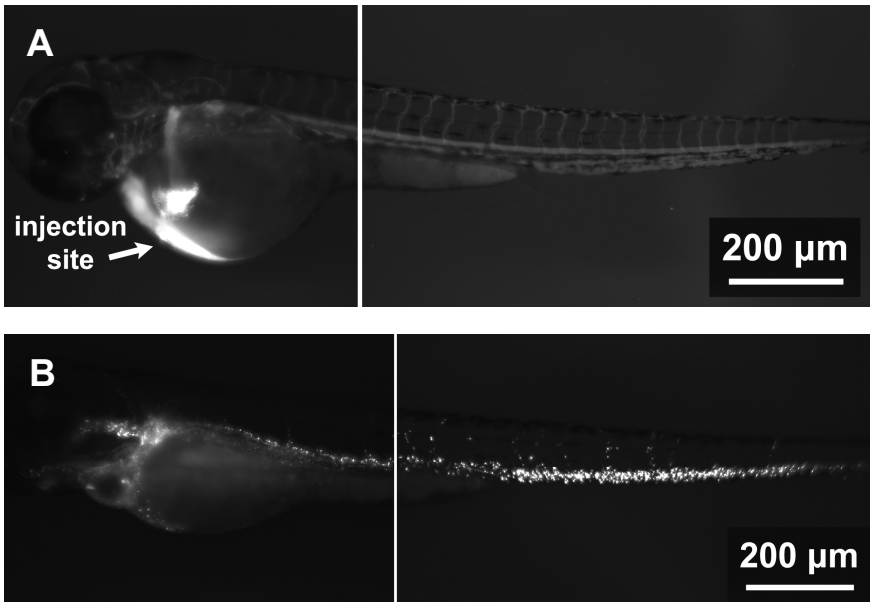


Figure S10. (A) Two (composited) epifluorescence images of a zebrafish embryo, 5 min after injection with CdSe@ZnS@PE-PEG-COOH (EviTag Type 2). Within 30 min, fluorescence from this embryo was indistinguishable from background. (B) Two (composited) epifluorescence images of a zebrafish embryo, 30 min after injection with CdSe@ZnS-S-PEG-COOH (EviTag Type 1). Fluorescence from these particles in the fish vasculature was qualitatively unchanged for two days after injection. In both cases, images were obtained with an ET-DSRed filter set (Chroma Technology, Rockingham, VT).

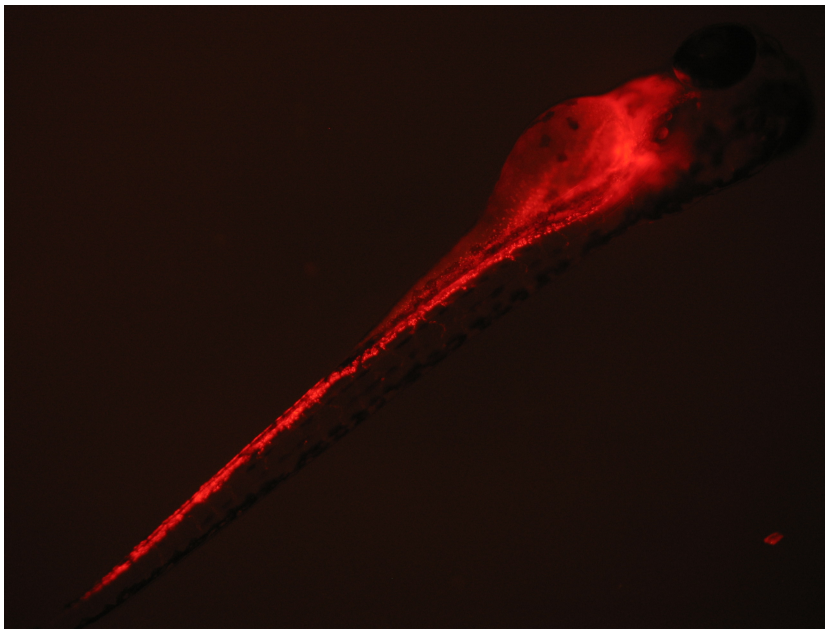


Figure S11. Epifluorescence (real-color) image of a zebrafish embryo, 5 min after injection with CdSe@ZnS@PS-*b*-PAA$_{25\%XL}$. Image was obtained with an ET-DSRed filter set (Chroma Technology, Rockingham, VT).

References

(1) Kang, Y.; Taton, T. A. *Angew. Chem., Int. Ed.* **2005**, *44*, 409.
(2) Westerfield, M. *The zebrafish book: A guide for the laboratory use of zebrafish (Danio rerio).* University of Oregon Press: Eugene, OR, 2000.
(3) Kimmel, C. B.; Ballard, W. W.; Kimmel, S. R.; Ullmann, B.; Schilling, T. F. *Dev. Dyn.* **1995**, *203*, 253.
(4) Nasevicius, A.; Larson, J.; Ekker, S. C. *Yeast* **2000**, *17*, 294.