Supporting Information for

Quantum Dot Nanotoxicity Assessment Using the Zebrafish Embryo

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Supporting Information is 6 pages (including the cover sheet) with a description of the sources and purities of chemicals used, details regarding QD synthesis (and references), 1 Table, and 2 supplementary figures.

Experimental Section

Chemicals. Cadmium chloride hemipentahydrate ($CdCl_2$, Sigma Aldrich, >98% pure), selenium tetrachloride ($SeCl_4$, Fisher, >98% pure), zinc chloride ($ZnCl_2$, Sigma Aldrich, >98% pure), poly-L-lysine (Sigma Aldrich, 99% pure), PEG_{350}-OCH_3 (Diagnostic Chemicals LtD, >95% pure) and PEG_{5000} –COOH, –OCH_3 or –NH_3 (RAAP Polymere, >96% pure) were used as obtained. Stock solutions were prepared in distilled deionized water (ddH_2O; 18 MΩ-cm resistivity).

Quantum Dot Functionalization. Mercaptoproprionic acid (MPA), PEG_{350}-OCH_3, PEG_{5000}–COO^-, PEG_{5000}–OCH_3, or PEG_{5000}–NH_3 were added via a ligand exchange process (*1,2*). Poly-L-lysine was added to MPA ligands using a charge-stabilized hydrogen bonding polymer wrapping procedure (*3*). After functionalization and removal of residual solvent by evaporation, QDs were suspended in ddH_2O and filtered through a 0.45-μm syringe filter.

Calculation of Expected QD diameters (d_{calc}). The first exciton peaks of the QDs were centered between at 558 nm for the PLL-wrapped QDs and between 525 and 530 nm for PEGylated QDs, corresponding to 3.2 and 2.6 nm, respectively (*4,5*). X-ray photoelectron spectroscopy indicated that the ZnS did not exceed 1 nm diameter (*6*). Core-shell diameter was confirmed by TEM (*6*). The thickness of the PLL wrapping was calculated from bond lengths assuming a single PLL shell with both the MPA and PLL in fully extended conformation. Application of an empirical relationship between effective diameter and molecular mass for free PEG molecules (*7*) indicated that the length of attached PEG molecules was 1 nm for PEG_{350} and 4.5 nm for PEG_{5000}. Calculated diameters (d_{calc}) of individual PLL-wrapped and PEGylated QDs (based on estimated contributions of the CdSe core, ZnS shell and PLL coating/extended PEG ligands) are presented in Table 1.

SI Literature Cited

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Table S1. Individual Correlations of Cd Body Burden with Toxicity Score and MT Fold Induction in Zebrafish Exposed to $CdCl_2$ or Quantum Dots.

	Toxicity Score			**MT Fold Induction**		
	R^2	**slope**	***p***	R^2	**slope**	***p***
$CdCl_2$	0.90	0.5	0.04	0.99	0.189	<0.001
$CdSe_{core}/ZnS_{shell}$-PLL	0.68	1.9	0.50	0.02	0.024	0.12
$CdSe_{core}/ZnS_{shell}$-PEG_{350}-OCH_3	0.10	0.01	0.61	0.27	0.159	0.48
$CdSe_{core}/ZnS_{shell}$-PEG_{5000}-NH_2	0.98	0.2	0.009	0.99	0.167	0.003
$CdSe_{core}/ZnS_{shell}$-PEG_{5000}-COO^-	0.97	0.3	0.01	0.002	0.002	0.96
$CdSe_{core}/ZnS_{shell}$-PEG_{5000}-OCH_3	0.95	0.2	0.01	0.96	0.340	0.02

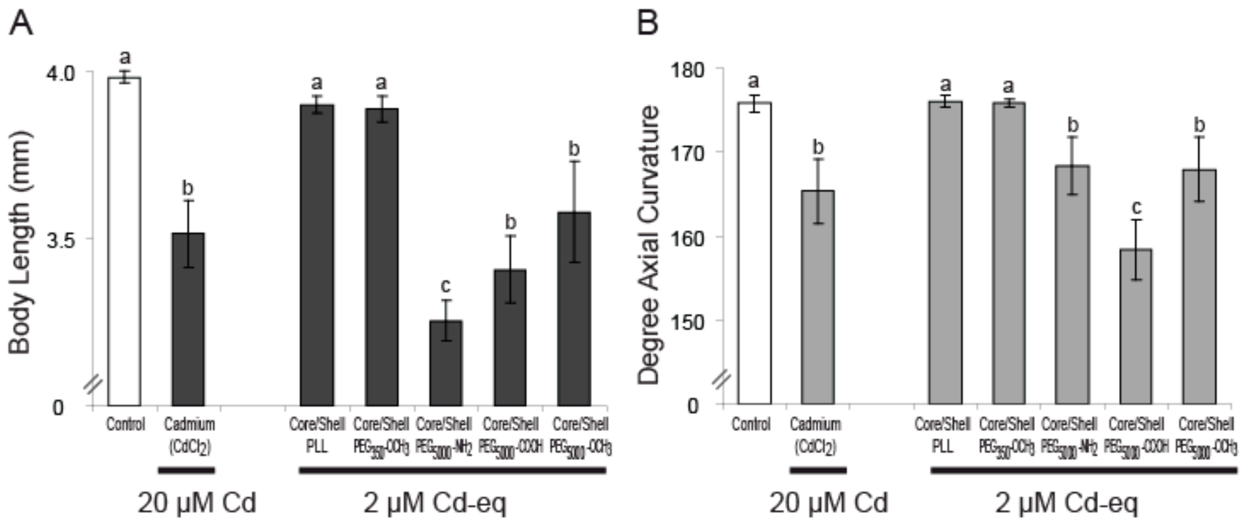

Figure S1. Exposure to an equally effective, mortality-inducing concentration (3-6% mortality) of QDs or $CdCl_2$ for 120 h (A) decreased zebrafish larvae growth, and (B) increased the curvature of bent spines. Values represent mean ± SE (n = 12); letters denote significant differences across treatments ($p \leq 0.05$). Cores were composed of CdSe; shells were ZnS.

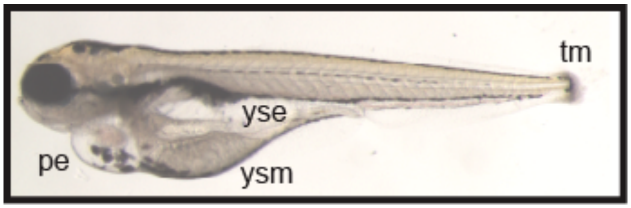

Figure S2. Representative photomicrograph demonstrating toxicity induced in zebrafish larvae by 120-h exposure to ≥ 200 μM selenium (as $SeCl_4$). Abbreviations: pe = pericardial edema; yse = yolk sac edema; ysm = yolk sac malformation (reduced absorption of yolk and opaque tissue indicating apparent necrosis); tm = tail fin malformation.