Supporting Information

Oxidative Quenching and Degradation of Polymer-Encapsulated Quantum Dots: New Insights into the Long Term Fate and Toxicity of Nanocrystals In-Vivo

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Methods

Chemical reagents. Reagent-grade NaOCl, HPLC-grade water, NaH_2PO_4 (98-102%), Na_2HPO_4 (98-102%), NaOH (>98%), volumetric-grade $Na_2S_2O_3$, KI (99+%), Chelex 100 resin (sodium form), phorbol 12-myristate 13-acetate (PMA), hydrogen peroxide (30% w/v, A.C.S. reagent grade) and dimethyl sulfoxide (DMSO, molecular biology grade) were purchased from Sigma-Aldrich. 2-mercaptoethanol (sold as ß-mercaptoethanol) was purchased from Bio-Rad. HOCl was prepared fresh daily by dilution of reagent-grade NaOCl (Aldrich) into HPLC-grade water (Sigma-Aldrich) at approximately 20 mM final concentration. HOCl concentration was measured both by iodometric titration with $Na_2S_2O_3$ and by UV absorbance in basic conditions. For UV-vis measurements, ε_{292nm} was taken to be 350 $M^{-1}cm^{-1}$.[1] Phosphate buffer stocks were made from NaH_2PO_4 and Na_2HPO_4 diluted into HPLC-grade water, with a final pH adjustment to 7.4 made with 1.0 M NaOH. The final phosphate concentration of the buffer was 1.0 M. The phosphate buffer stock was treated overnight with Chelex 100 resin (Sigma-Aldrich) to remove contaminating transition metal ions that could interfere with downstream ICP-MS analysis.[2]

Quantum dot synthesis and solubilization. For all experiments, the same batch of colloidal CdSe/CdS/ZnS quantum dot nanocrystals (QDs) was used. The QDs were synthesized as detailed elsewhere.[3] For the QDs used in these experiments, the QD core (CdSe) was estimated to be 3.4 nm in diameter, with an estimated 1.75-nm thick shell (CdS/ZnS), for an overall size of 6.9 nm in diameter. The measured diameter of the QDs in $CHCl_3$ was 7.0 nm by dynamic light scattering (DLS). The final QDs had an emission peak at 618 nm with a full-width-at-half-maximum of 22 nm and an approximate quantum yield of 60%. The QD were phase transferred into water following published methods.[3,4] The PAA-*g*-DDA polymer was similar to that used by Wu et al.,[5] but used dodecylamine in place of octylamine. See the Supporting Table 1 for detailed information of various quantum dots synthesized and used in this work.

Photoluminescence measurement and photoannealing. PAA-*g*-DDA encapsulated QDs were diluted in 2 mL glass vials to a final concentration of 50 nM into 0.1 M phosphate-buffered HPLC-grade water with 0-500 µM HOCl present. The QDs were gently mixed by inverting, and allowed to rest 30 minutes in the dark before measuring photoluminescence (PL) intensity in a fluorometer (PTI) with an excitation light of 470 nm and a slit width of 2 nm. QDs were photoannealed by exposure to UV-B light on a UV light stand (UVP XX15-M, with two 15-W 306 nm bulbs) at a working distance of 6 cm for 10 minutes. The QDs were allowed to rest 30 minutes in the dark before measuring PL intensity.

Fluorescence imaging of QD vials. QDs were imaged approximately 35 cm from a Nikon D100 with a 60 mm f/2.8 lens and a UV filter (Quantaray MC UV), with illumination provided by a 15W fluorescent black light (GE F15T8 BLB) in a darkened room. Images were acquired in

RAW mode with an ISO sensitivity of 400, f/22, and a 6 second exposure. White balance was applied post-imaging with Raw Photo Processor (Andrey Tverdokhleb, v 3.7.0 on Apple OS X v. 10.5.2) using the automatic setting.

UV-Vis absorption measurement. PAA-*g*-DDA encapsulated QDs diluted to 0.5 µM were reacted with 0.5 µM-5 mM HOCl in 0.1 M pH 7.4 phosphate buffer in the dark at room temperature for 30 minutes. The UV-vis spectra were then recorded with a Shimadzu UV-2401PC.

Inductively coupled plasma – mass spectrometry (ICP-MS). PAA-*g*-DDA encapsulated QDs diluted to 50 nM were reacted with 0.5 µM-5 mM HOCl in 0.1 M pH 7.4 phosphate buffer in the dark at room temperature for 1 week. The QD medium was then eluted through a 10 k MWCO Microcon centrifugal ultrafiltration membrane (Millipore) and submitted for ICP-MS analysis at the CAIS facility at UGA.

Stimulated HL-60 cell QD fluorescence quenching. The HL-60 cell line was obtained from ATCC (accession number CCL-240) and cultured in RPMI media supplemented with 20% fetal bovine serum and 1% penicillin/streptomycin (all obtained from ATCC) in a 37ºC incubator with 5% CO_2 as per ATCC recommendations. To differentiate HL-60 cells into a neutrophil-like phenotype, the cell growth medium was supplemented with 1.3% DMSO, in which the cells were cultured for 7 days.[7] The terminally-differentiated neutrophil phenotype of the HL-60 cell line undergoes an oxidative burst when stimulated which includes the production and release of hypochlorous acid.[8] Terminal cellular differentiation was verified by cessation of proliferation, change in cell size, and band neutrophil staining pattern with the Wright-Giemsa stain. For stimulated quenching experiments, differentiated HL-60 cells were transferred from growth medium into Hank's buffered saline solution (HBSS) with calcium and magnesium but without phenol red by centrifugation. The reaction was carried out in a black 96-well microtiter plate, with a final concentration of 1 nM PAA-*g*-DDA encapsulated QDs, 100,000 HL-60 cells, between 10 nM – 1 µM PMA in DMSO (or DMSO alone as a control), and 1 mM sodium azide (NaN_3), all with HBSS as a diluent. The cells were incubated at 37ºC for 1 hour following the addition of PMA, and then read in a fluorescence microplate reader (BioTek Synergy2, Winooski, VT) with a 360 nm excitation filter, 400 nm dichroic mirror, and 620 nm emission filter. The fluorescence intensity of cells alone was taken to be the background signal and subtracted from all wells, and the fluorescence intensity of unstimulated cells with QDs was taken to be 100% relative fluorescence intensity for normalization.

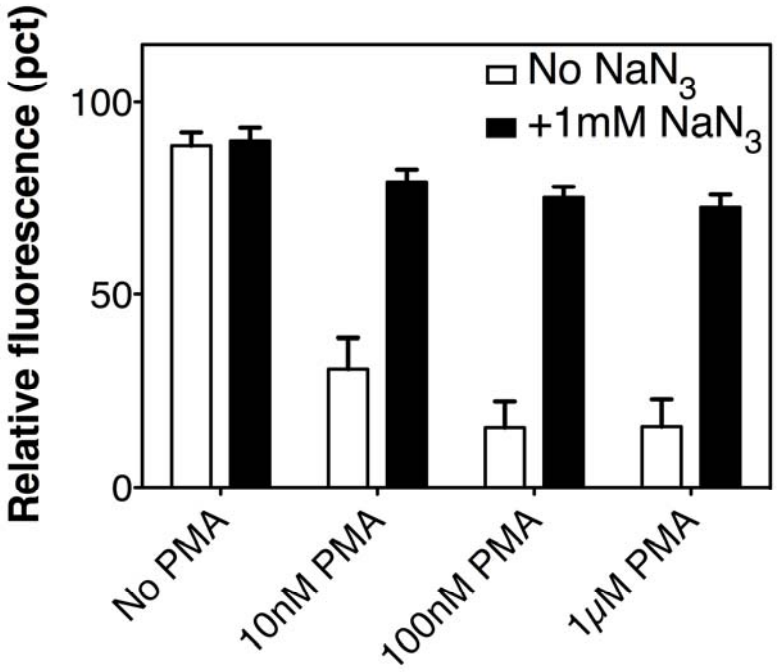
QD quenching by hydrogen peroxide. PAA-*g*-DDA encapsulated QDs were diluted to a final concentration of 10 nM in a 0.1M pH 7.4 phosphate buffer in a black 96-well microtiter plate (Nalge Nunc, Rochester, NY). H_2O_2 was added at a final concentration of 0.1 – 50 µM and was allowed to react with QDs for 0, 1, 24, and 48 hours. The microplate was stored in the dark at room temperature and covered with Parafilm in between additions of H_2O_2. The plate was read on a fluorescence microplate reader (BioTek Synergy2, Winooski, VT) with a 470 nm excitation filter, 515 nm dichroic mirror, and 620 nm emission filter.

Effect of mercaptoethanol on QD quenching. PAA-*g*-DDA encapsulated QDs were diluted to 10 nM in a 0.1M pH 7.4 phosphate buffer in the presence or absence of 1 µM 2-mercaptoethanol in a black 96-well microtiter plate (Nalge Nunc, Rochester, NY). 10 nM – 50 µM HOCl was then added to the QD solution, the microplate was vortexed, and allowed to rest 30 minutes in the dark. The plate was then read on a fluorescence microplate reader (BioTek Synergy2, Winooski, VT) with a 470 nm excitation filter, 515 nm dichroic mirror, and 620 nm emission filter.

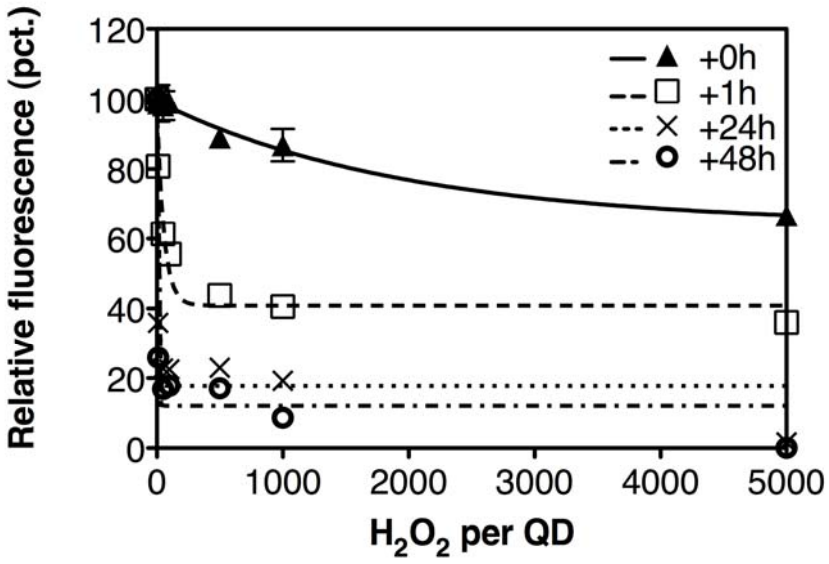
Supporting Tables and Figures

Supporting Table 1. **Summary of chemical composition, size, and optical properties of semiconductor QDs used in this work.** The QD diameter was determined based on the sizing curves of Yu et al.[6] The number of atoms per core/shell QD was estimated by assuming complete reaction of the elemental precursors added to the reaction. The final fluorescence emission peak wavelength and the spectral width (full-width-at-half-maximum or FWHM) were 619 nm and 22 nm, respectively.

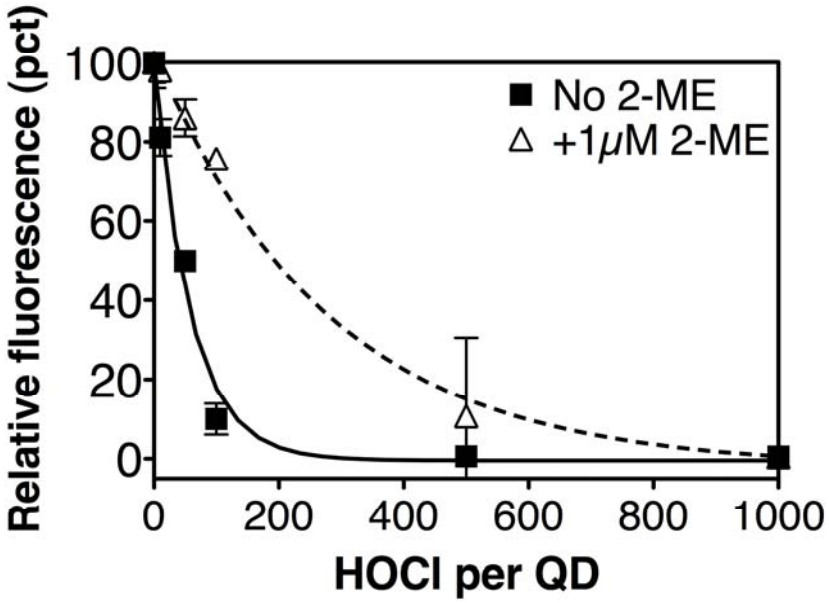
Monolayer Number	Material	Approx. Diameter (nm)	Approx. Atoms per QD	Fluorescence peak (in $CHCl_3$)	FWHM (nm, in $CHCl_3$)
0	-	3.36	468	572.9	23.7
1	CdS	4.06	826	580.6	25.8
2	CdZnS	4.76	1333	586.3	26.5
3	CdZnS	5.46	2013	595.6	23.1
4	CdZnS	6.16	2892	606.0	22.0
5	ZnS	6.86	3995	615.8	20.8



Supporting Figure 1. **Quenching of QD fluorescence by neutrophil-like HL-60 cells after oxidative stimulation.** The human promyelocytic leukemia cell-line HL-60 was differentiated into a neutrophil-like phenotype by co-culture with DMSO.[7] The cells were then stimulated with phorbol 12-myristate 13-acetate (PMA) to generate "oxidative bursts", leading to extracellular and intraphagasomal release of hypochlorous acid (HOCl).[8] Sodium azide (NaN_3) was used as an inhibitor of the enzyme myeloperoxidase in HOCl production.[9] When co-incubated with QDs, the differentiated HL-60 caused QD fluorescence quenching in a PMA dose-dependent manner. The addition of NaN_3 greatly reduced the amount of QD fluorescence quenching for all doses of PMA. Error bars show the standard deviations (n = 4).



Supporting Figure 2. **QD fluorescence quenching by hydrogen peroxide at various H_2O_2/QD ratios and exposure times.** Polymer-encapsulated QDs (10 nM) were exposed to 0.1-50 µM H_2O_2 at the physiologic pH (7.4). QD fluorescence quenching by H_2O_2 was kinetically slower than that induced by HOCl exposure, in agreement with their differences in chemical reaction rates. Error bars show the standard deviations (n = 4), and the regression lines are a non-linear fit of the data to an exponential decay model.



Supporting Figure 3. **Protective effect of free mercaptoethanol on fluorescence quenching of quantum dots by hypochlorous acid.** The inclusion of 1 µM 2-mercaptoethanol (2-ME), equivalent to 100 2-ME per QD, reduced the loss in fluorescence of QDs following hypochlorous acid (HOCl) exposure. Once the amount of HOCl added exceeds the amount of 2-ME present (at 100 HOCl per QD), the effect of 2-ME in preventing QD fluorescence quenching is minimal. Error bars show the standard deviations (n = 4), and the regression lines are a non-linear fit of the data to an exponential decay model.

Supporting References

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