Supporting Information for

Detection of toxic mercury ions using a ratiometric CdSe/ZnS nanocrystal sensor

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Experimental Methods

All chemicals were obtained from commercial sources and were used as received unless noted.

Instrumentation: ^{1}H NMR spectra were recorded on a Bruker Avance DRX 400 NMR Spectrometer. UV-Vis absorbance spectra were taken using a Varian Cary 300 Bio UV-Vis Spectrophotometer. Fluorescence emission spectra were taken using a Fluoromax-3 (HORIBA Jobin-Yvon). The absorbance of all solutions was kept near or below 0.1 OD at the excited wavelength to avoid inner-filter effects.

NC Synthesis: Core CdSe and core shell CdSe/ZnS NCs were synthesized according to previously published protocols.[1-2] Samples were processed by addition of a small amount of isopropanol followed by methanol to precipitate the samples. The supernatant is discarded leaving behind a gel most likely composed of a decylamine-tetradecylphosphonic acid adduct used in the ZnS overcoating procedure. The CdSe/ZnS NCs were extracted from this gel by several washings of hexane; the washings were collected from which the NCs were precipitated again through the addition of a few drops of isopropanol and sufficient methanol. The precipitate was collected by centrifugation and dried under reduced pressure. These NCs were water solubilized with a 40% octylamine modified poly(acrylic acid) polymer, see ref. 3 for details on the synthesis and characterization.

Rhodamine B thiosemicarbazide synthesis: A mercury sensitive thiosemicarbazide functional dye was synthesized from a protocol developed by Yang et al.[4-5] We have used rhodamine B as the dye substrate due to its spectral overlap with the ~550 nm emitting water-soluble CdSe/ZnS NCs our lab routinely produces. First, 300 mg of rhodamine B and 0.10 mL of hydrazine monohydrate were refluxed together in methanol for 6 hours under a nitrogen atmosphere. After the reaction was cooled to room temperature, 30 mL of ethyl acetate was added and the organic and aqueous layers were separated. The organic layer was dried and the residuals packed into a chromatography column with silica gel using hexanes. The product was eluted using a 10:2:1 mixture of hexane, dichloromethane, and methanol. Next, 27 mg of the isolated rhodamine-hydrazide product and 80 mg of phenyl isothiocyanate are stirred together in 2 mL of DMF overnight. After drying, the product was purified over silica gel by column chromatography using a 4:1:1 ratio of hexane, dichloromethane and ethyl acetate mobile phase. The product can be stored in the refrigerator for several weeks; ^{1}H NMR data ($CDCl_3$) on this compound is provided below. The product is not stable in solution and will return to a visible absorptive / emissive state within days.

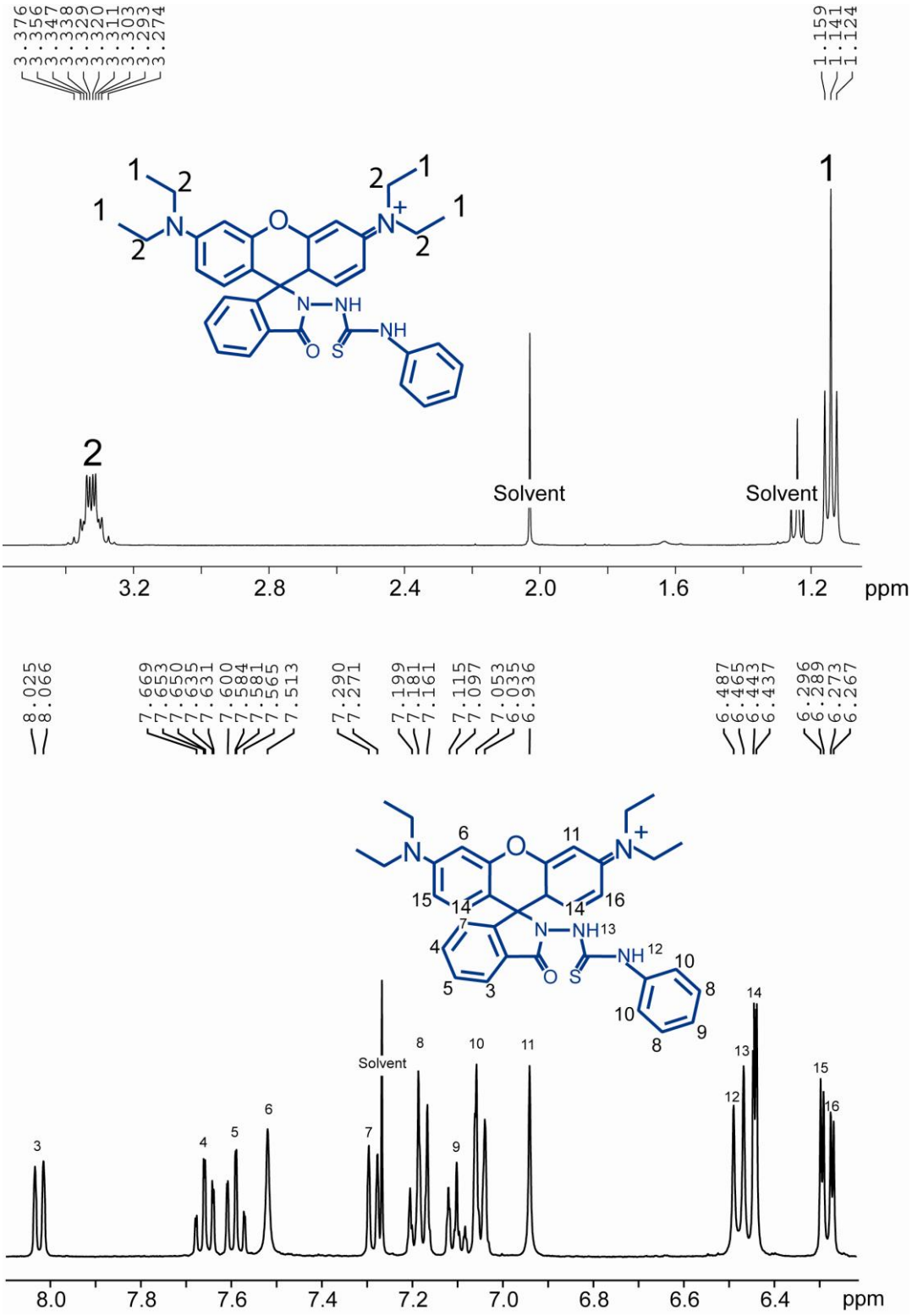

Figure S1. ^{1}H NMR spectrum of the thiosemicarbazide-functional rhodamine B probe developed in this study. COSY was also used to verify the assignments in the aromatic region. Residual ethyl acetate (solvent) is also visible in the spectra.

Hg^{2+} Sensor: To make a fluorescent Hg^{2+} sensor, the rhodamine-based probe is dissolved in DMF and added in subsequent ~sub-milligram dye quantities to the water-soluble NCs in increasing amounts. After stirring overnight, the samples were dialyzed using high molecular weight cutoff concentrating filters from Millipore (Amicon Ultra, 100K cut-off) with deionized water. The rinse was checked for the presence of mercury-sensitive dye to assure that the dye-NC complexes were properly isolated from freely soluble dye. No dye was observable in the washings indicating that non-specific interactions between the hydrophobic dye and NCs are very strong. As observed with the neat dye, the dye component converts to an absorptive / emissive state over the course of several days. As such, optical characterizations were performed over the course of a few hours such that the degradation of the dye does not affect the results.

Optical Characterization: To measure the emission of the of the dye-NC sensors as a function of Hg^{2+} ion exposure, 2.5 mL of a ~1.5 μM NC solution containing dye-to-NC ratios of ~41 and ~11 were placed inside the optical cavity of a Fluoromax spectrofluorometer. The emission of the coupled chromophores was monitored as several 25 μL portions of a 2.1×10^{-4} M of $HgCl_2$ (or 2 μL portions of a more concentrated 5.8×10^{-3} M solution) were added; the mercury standards were prepared immediately before use. The emission of aqueous NCs as well as thiosemicarbazide functional dye solubilized in 40% octylamine-modified poly(acrylic acid) were measured as a function of mercury content. All emission spectra were corrected for the absorptivity of the chromophores as well as the wavelength-dependent instrument sensitivity.

Detection Limits: To determine the limit of detection (LOD), we employed the bootstrap method[6] to calculate the variation in the blank response by simulating 10,000 possible blank spectra, calculating the ratio of NC : dye emissions of each to determine the standard deviation (STD) of the blank response, and calculated the LOD as three times the STD divided by the slope of the linear calibration. The LOD of the neat dye was calculated in a similar manner. All numerical operations were performed using Matlab.

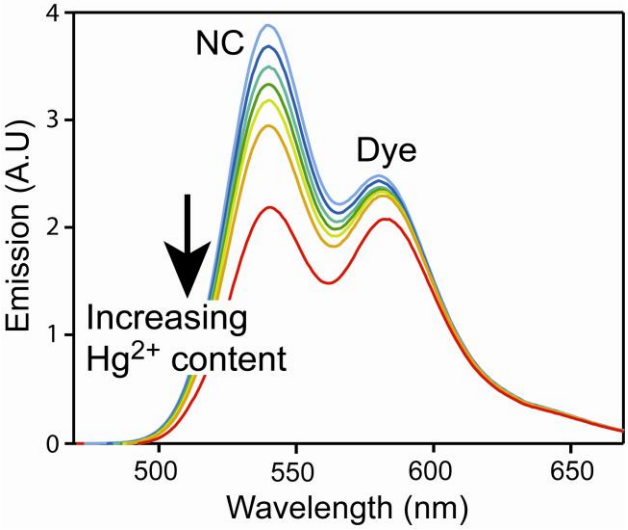

Figure S2. The emission spectra from an aqueous dye-NC coupled chromophore (dye:NC ratio = 41; blue=0 M → red=4×10^{-5} M) as a function of Hg^{2+} content; the normalized data are shown in Fig. 2 of the main text.

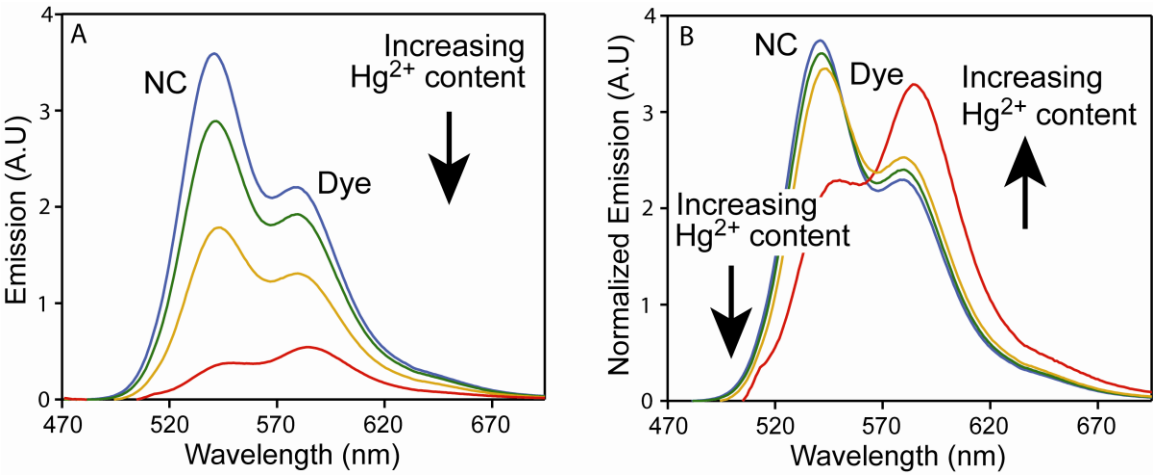

Figure S3. A. The emission spectra from an aqueous dye-NC coupled chromophore (dye:NC ratio = 11; blue=0 M → red=19×10^{-5} M) as a function of Hg^{2+} content. **B.** The same after normalization by the area reveals the ratiometric response to mercury.

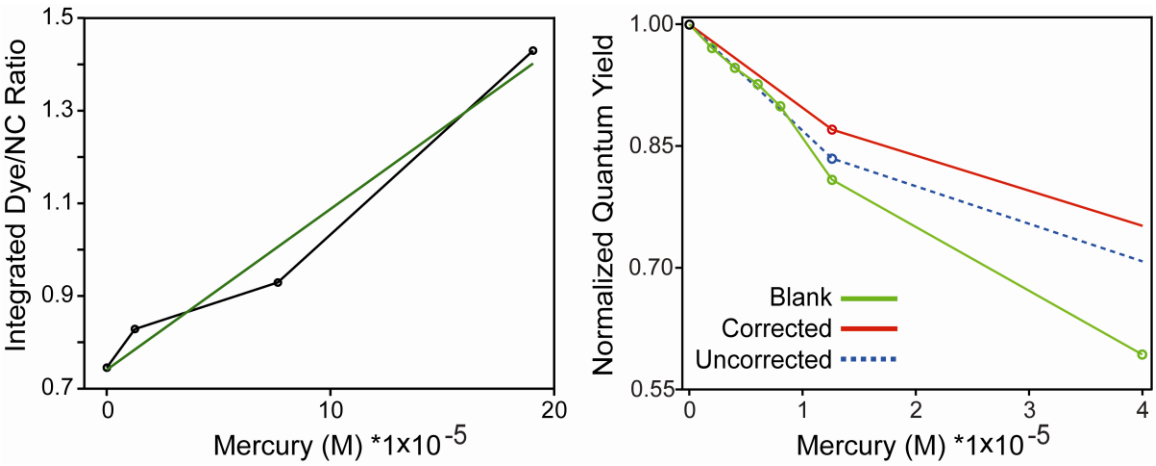

Figure S4. A. The integrated dye/NC emission ratio as a function of increasing mercury ion content for the sample of the lowest dye-loading ratio (dye:NC ratio = 11, slope = 3400±400 M^{-1}). Note that this slope is 0.21× less than that observed with the more coupled dye:NC (ratio = 41) sensor; this implies that the responsivity scales linearly with the dye:NC ratio. **B.** The relative quantum yields of unconjugated "blank" NCs and coupled dye-NC chromophores as a function of addition of aqueous Hg^{2+}. The green line represents the response of the blank NCs; also shown are the normalized integrated emissions of dye-coupled NCs with correction for the finite quantum yield of the organic component (red) and without (blue-dash). The level of NC "protection" is less than that observed with the sample with the largest dye:NC ratio. The detection limit was calculated to be 160 ± 20ppb, which is also less than that observed with the more coupled sample (79 ± 2 ppb).

Metal Selectivity: The thiosemicarbazide functionality of a rhodamine dye was shown to be significantly more reactive towards the presence of mercury compared to a host of other metal ions in the original report by Yang et al.;[5] we demonstrate here that the selectivity is preserved in our system when the NC-dye sensor is exposed to a host of aqueous metal solutions of identical concentrations. While some dye activation is observed in all the spectra, the overall response to Hg^{2+} ions is over ~20× times greater.

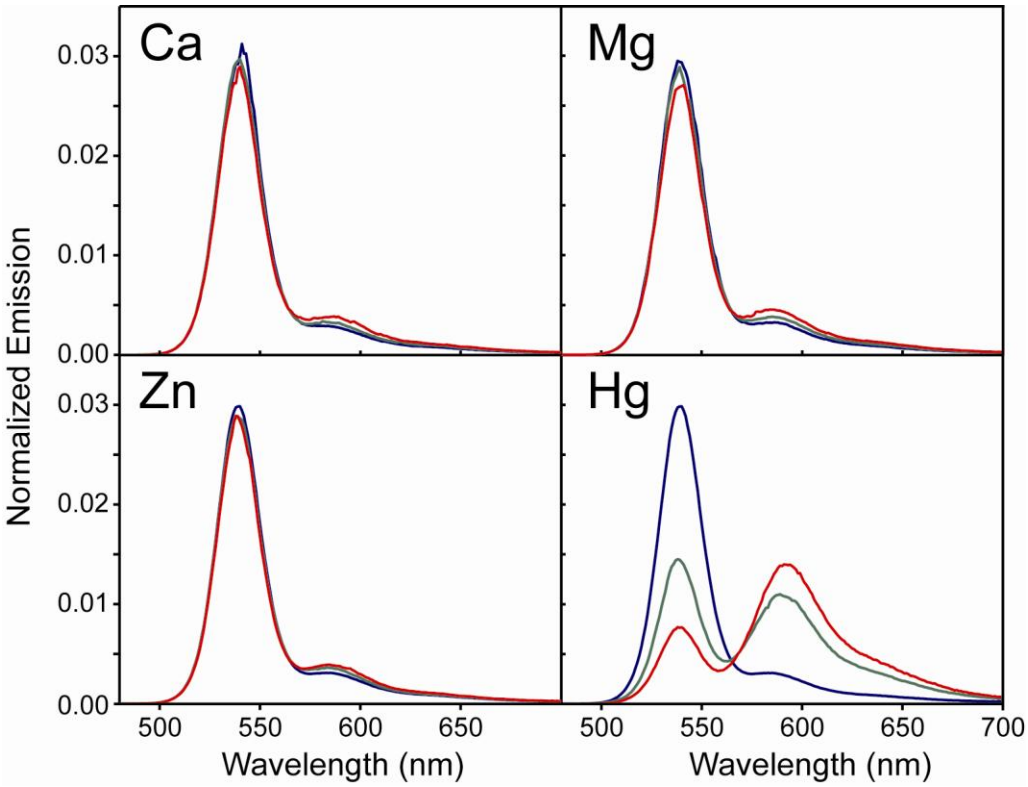

Figure S5. The normalized emission spectra from aqueous thiosemicarbazide-functional rhodamine B – CdSe/ZnS NC coupled chromophores (blue) exposed to an increasing level (green→red) of metal-salt analytes demonstrate the ratiometric-sensing specificity of our system towards mercuric ion exposure.

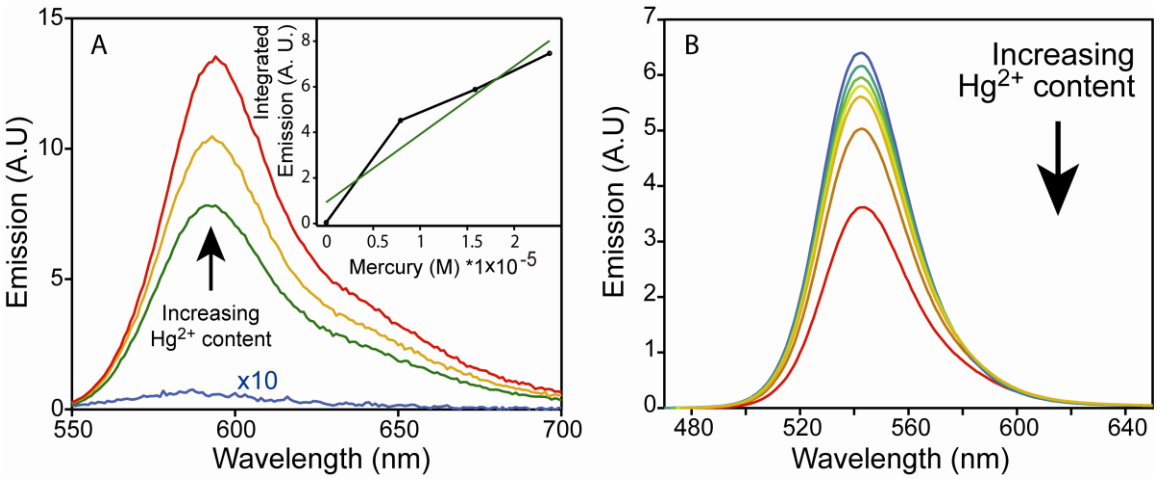

Figure S6. A. The emission spectra from mercury sensing rhodamine B dye a function of Hg^{2+} content (blue=0 M → red=2.375×10^{-5} M). Inset: calibration curve used in the determination of the LOD (slope=$3.0\pm0.6\times10^{9}$ Area(A.U.)·M^{-1}) **B.** The emission spectra from neat NCs (1.37×10^{-6} M) as a function of mercury ion exposure (blue=0 M → red=4.0×10^{-5} M).

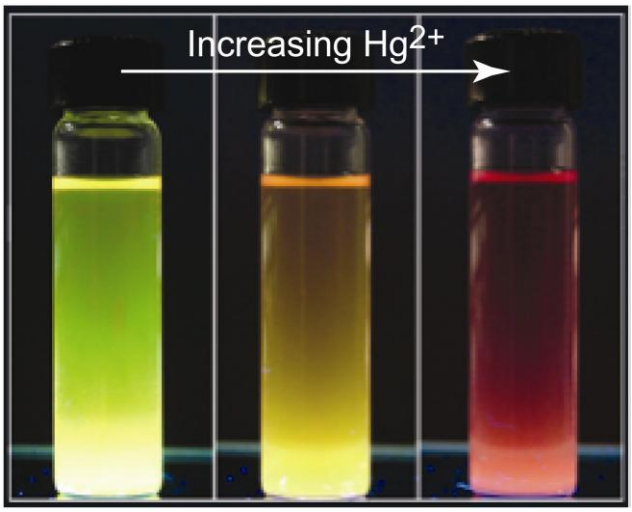

Figure S7. The emission of the sensing system is visible to the eye with addition of an aqueous Hg^{2+} solution, which was added until complete quenching of the NCs was observed.

FRET Efficiency Characterization: We have characterized the efficiency of energy transfer from the NC donor to the mercury sensing rhodamine B dye using photoluminescence excitation (PLE) spectroscopy. Shown in Fig. S7 are the spectra of the rhodamine derivative and NC-dye coupled chromophore monitored at 605 nm, where no NC emission exists. The dye spectrum (blue line) is weakest at 460 nm, the wavelength of excitation. The coupled chromophore (red line) has mostly nanocrystal-like features at higher energies as evident from the absorption spectrum of the neat NCs (black line). Furthermore, the emission of the dye component in the NC-dye coupled chromophore is over 12× stronger compared to that of the neat dye in water.

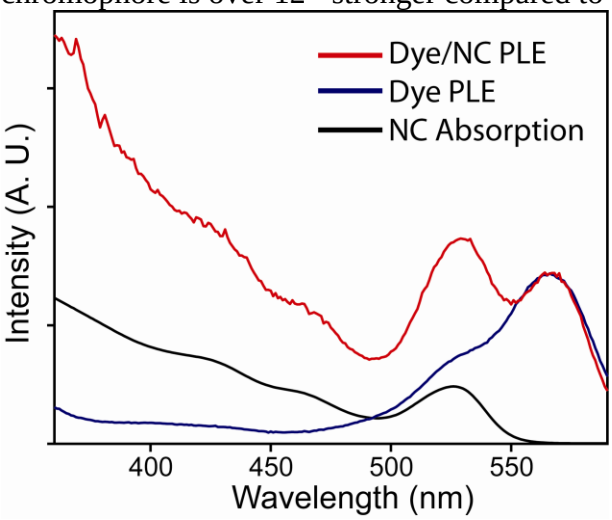

Figure S8. The photoluminescence excitation (PLE) spectra of the mercury-sensitive dye (blue) and NC-dye coupled chromophore (red) measured at 605 nm. The coupled

chromophore has strong CdSe/ZnS NC-like features below 550 nm as evident from comparison to the absorption spectrum of the neat NCs (black line).

A FRET distance of R_0=5.2 nm was calculated from the absorption spectrum of the mercury-activated dye, assuming a molar absorptivity of 97100 $M^{-1}cm^{-1}$ at the absorption maximum.[7] Several NC samples were used in this study; however one sample with a 540 nm emission (25 nm FWHM, QY: 30%) is fairly typical of the materials used in this study.

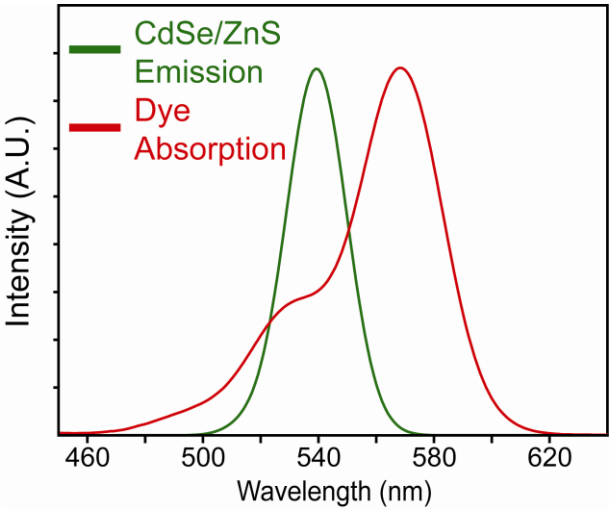

Figure S9. The overlap of the CdSe/ZnS NC emission (green) with the mercury-activated absorption (red) results in a characteristic FRET distance of R_0=5.2 nm.

References:

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