Supporting Information for

QD-based Fluorescent Ratiometric FRET Sensing for Bisulfide *in vitro*

Armen Shamirian,[a] Hamid Samareh Afsari,[a] Donghui Wu,[b] Lawrence W. Miller,[a] Preston T. Snee[a,] a. Department of Chemistry, University of Illinois at Chicago, 845 West Taylor Street, Chicago, Illinois 60607-7061, United States. b. Colgate-Palmolive Global Technology Center 909 River Road, Piscataway, NJ 08855-1343, United States.*

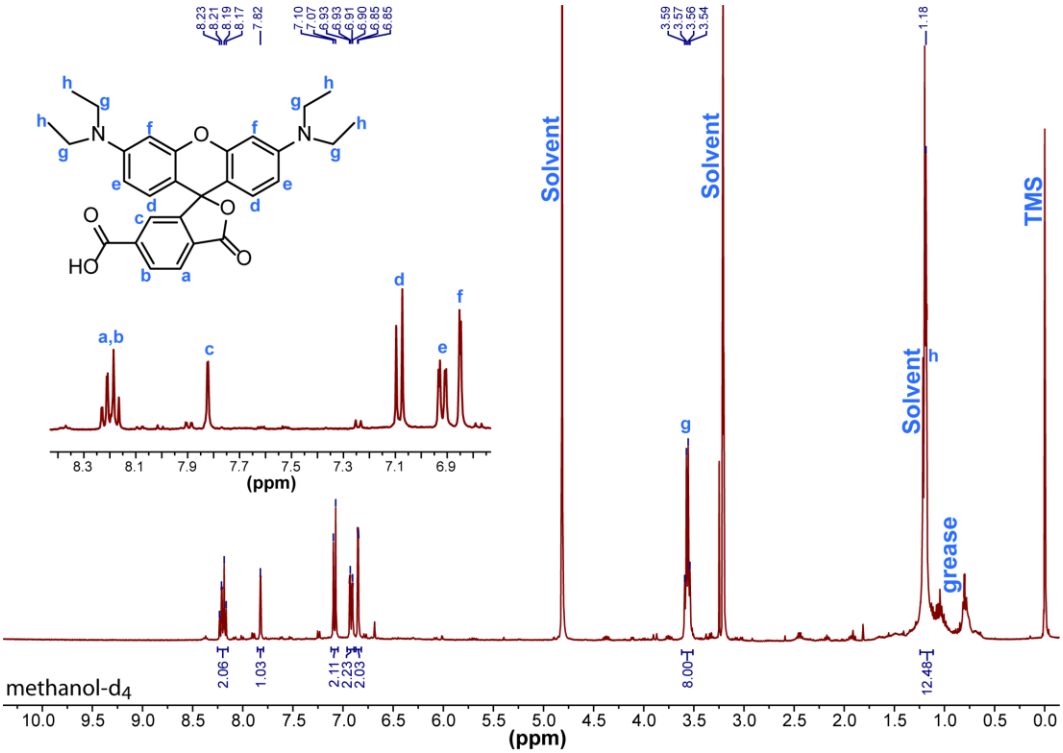


Figure S1. ^{1}H NMR spectrum of 6-carboxyrhodamine B (structure shown in the inset).

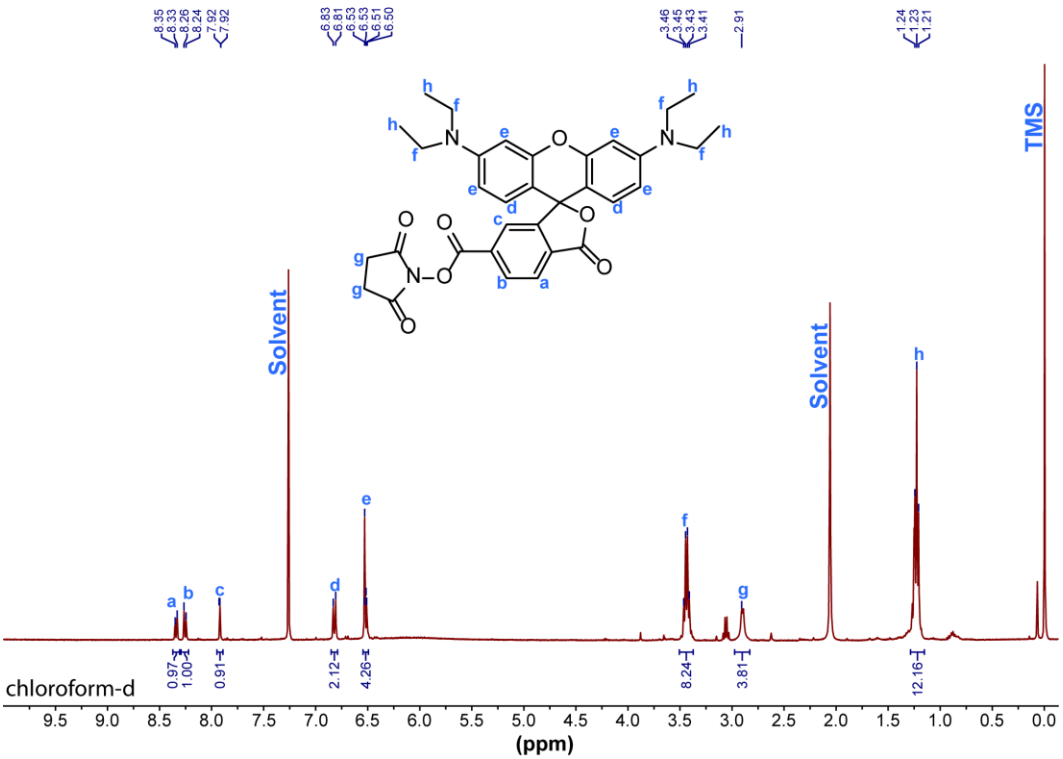


Figure S2. ^{1}H NMR spectrum of 6-carboxytetraethylrhodamine N-hydroxysuccinimide ester (structure shown in the inset).

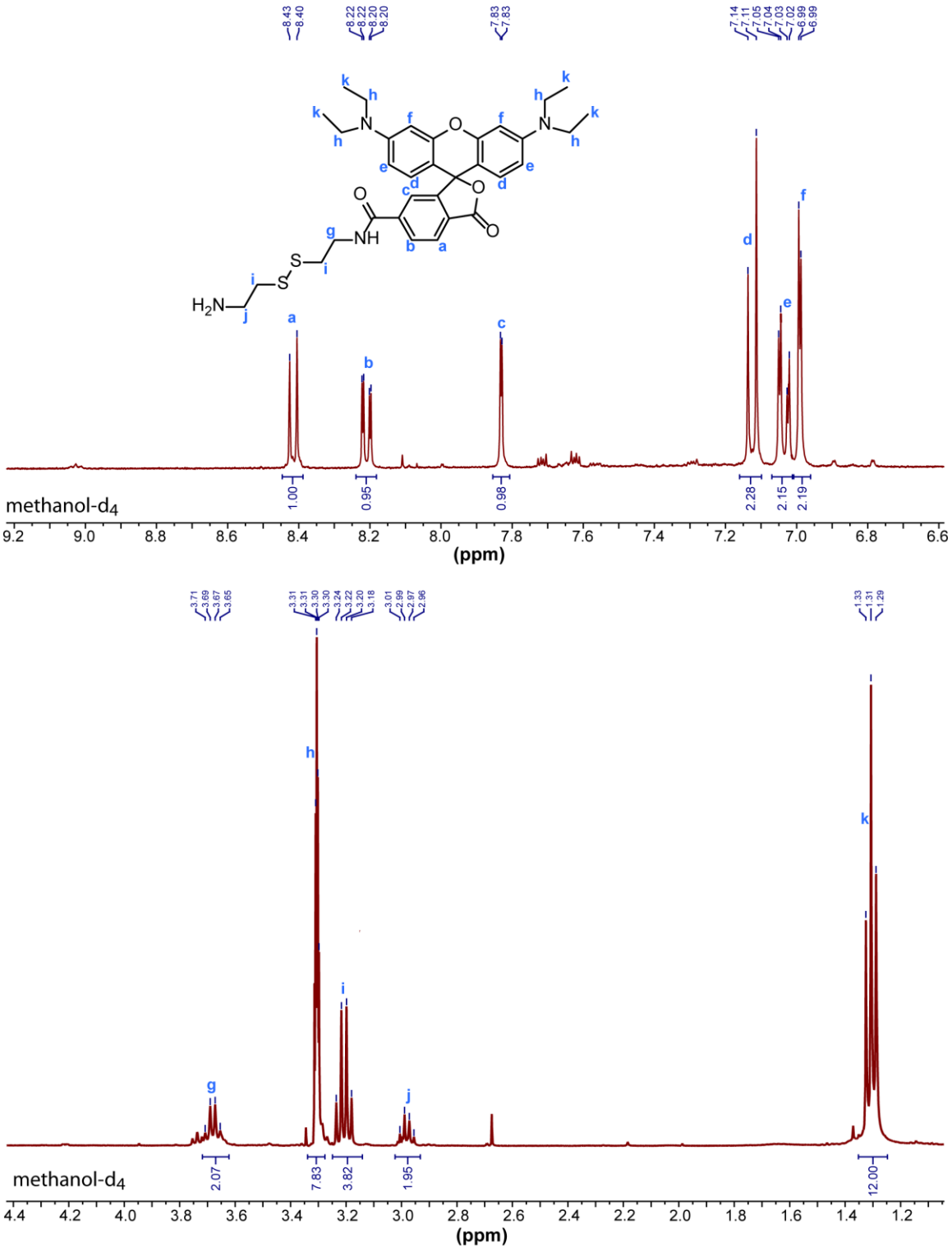


Figure S3. ^{1}H NMR spectrum of compound 8 in the aromatic (top) and aliphatic (bottom) regions (structure shown in the inset).

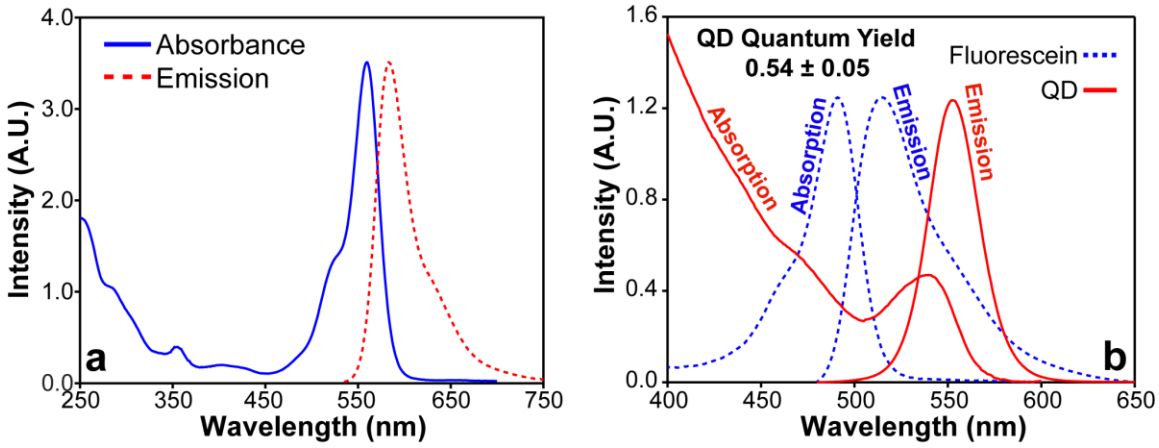


Figure S4. (a) Absorbance and emission spectra of the bisulfide-reactive carboxyrhodamine dye (compound 8). (b) The water-soluble CdSe/ZnS QDs used for the cell imaging / sensing studies have a quantum yield of 0.54 ± 0.05. Fluorescein in 0.1M NaOH was used as a standard (Ex. at 470 nm QY 0.91 ± 0.05), see Porres et al. *J. Fluoresc.*, **2006**, *16*, 267-272.

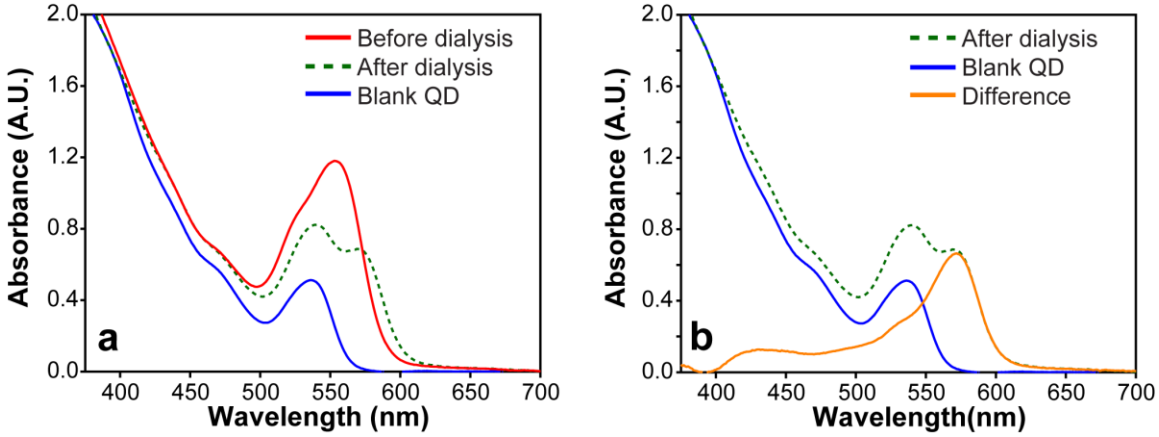


Figure S5. (a) The dye conjugation reaction yield (42%) was calculated from the ratio of the integrated dye absorption of the QD/bisulfide-reactive dye conjugate system before and after dialysis. (b) Subtraction of the blank QD from the QD-dye conjugate absorption reveals Rhodamine B dye-like features that were used to calculate of number of the dyes per QD.

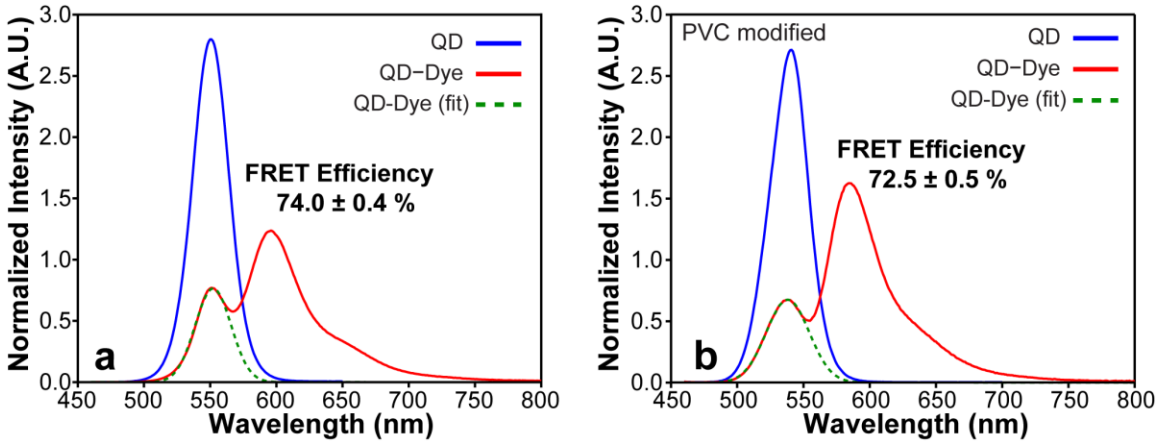


Figure S6. The FRET efficiency of the (a) unmodified and (b) PVC modified ("plastic coated") QD/bisulfide-reactive dye conjugates was determined by measuring the quenching of the donor only fluorescence and after conjugation to the energy accepting dye.

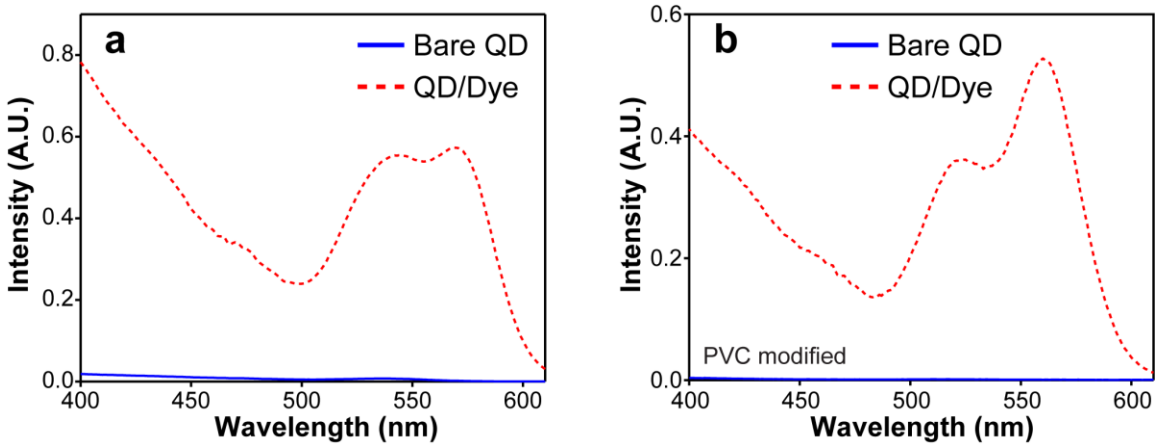


Figure S7. Photoluminescence excitation spectra of (a) water-soluble and (b) PVC modified water-soluble QDs (blue solid) and the same conjugated to the bisulfide-reactive carboxyrhodamine dye (red dash) measured by the intensity of emission at 620 nm, near the maximum of the dye fluorescence. The mixture of QD and dye-like features in the coupled chromophore and the relatively weak intensity of the neat QD sample are indicative of energy transfer from the QD to the dye.

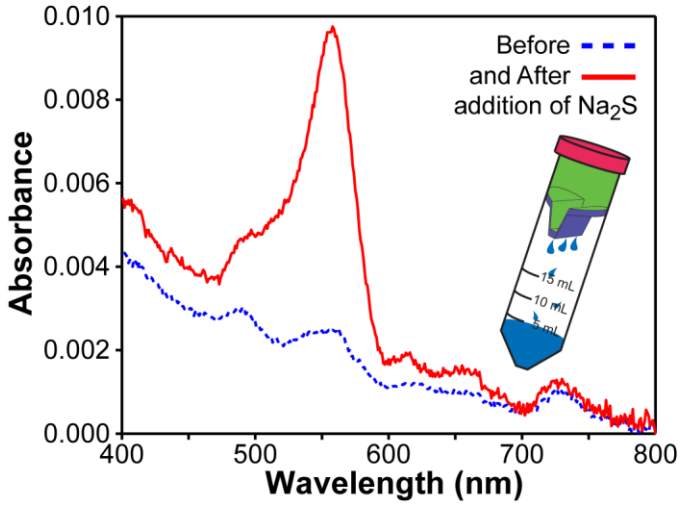


Figure S8. The absorption spectra of the filtrate from a QD/sulfide reactive dye coupled chromophore before and after exposure to Na_2S reveals that the presence of rhodamine dye in the filtrate after bisulfide exposure. This must be due to reduction of the S-S bond that links the quantum dot and dye.

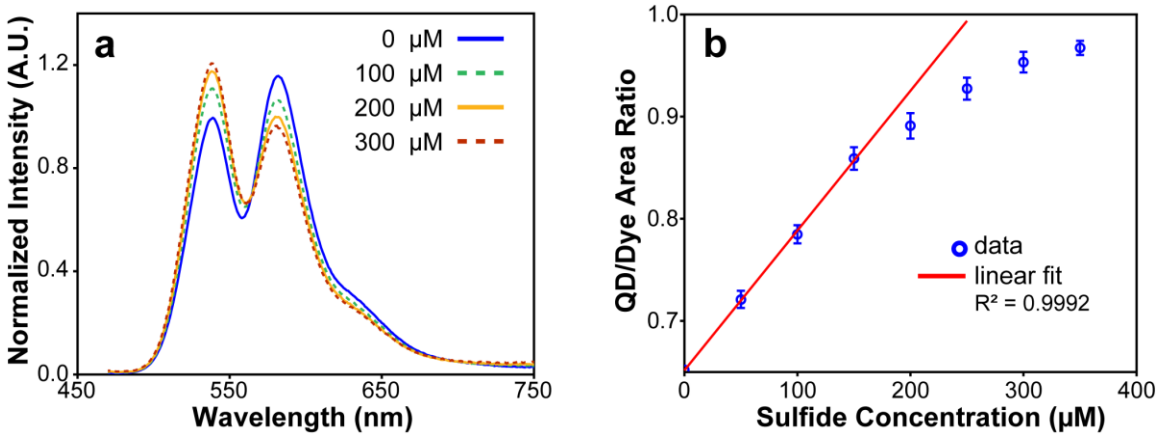


Figure S9. (a) Emission spectra of QD/rhodamine B piperazine conjugated chromophore in the presence of increasing concentrations of Na_2S. (b) Calibration data from the ratio of the integrated emission of the QD donor over the dye acceptor as a function of HS^- concentration reveal a roll-off of the response at higher levels of bisulfide that is not observed in the QD/ bisulfide-reactive carboxyrhodamine dye conjugate.

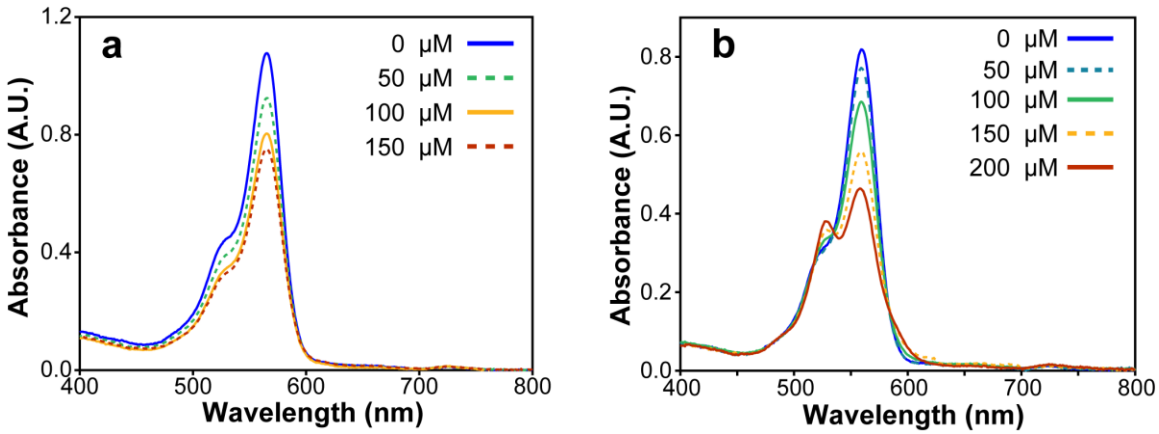


Figure S10. (a) Absorbance changes of Rhodamine B piperazine due to increasing exposure to Na_2S solution. (b) Absorbance changes of compound 8 due to increasing exposure to Na_2S solution.

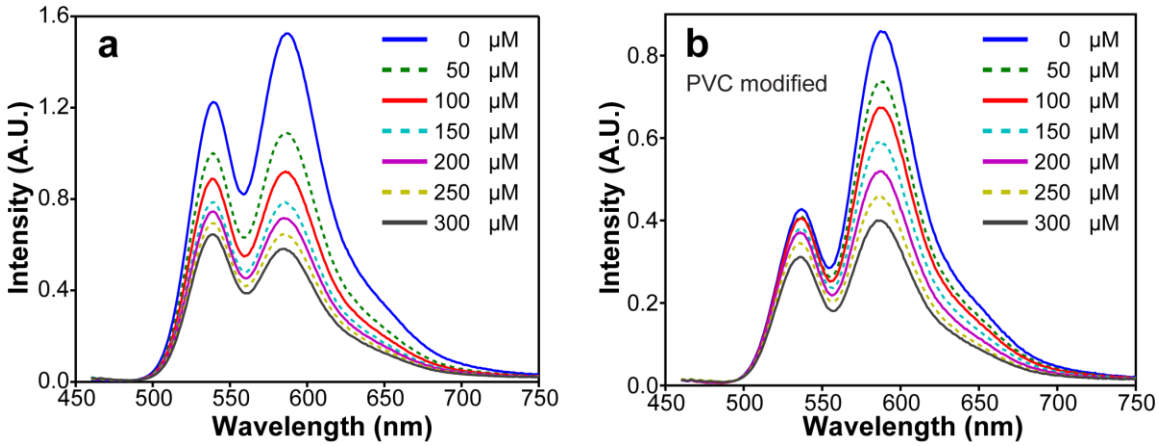


Figure S11. (a) Raw emission spectra of a) QD/sulfide reactive dye coupled chromophore, the normalized data from which appear in the main text (Figure 1a) and (b) PVC modified QD/sulfide reactive dye in the presence of increasing concentrations of Na_2S. The data show that the quantum dot emission is less perturbed in the plastic coated sample.

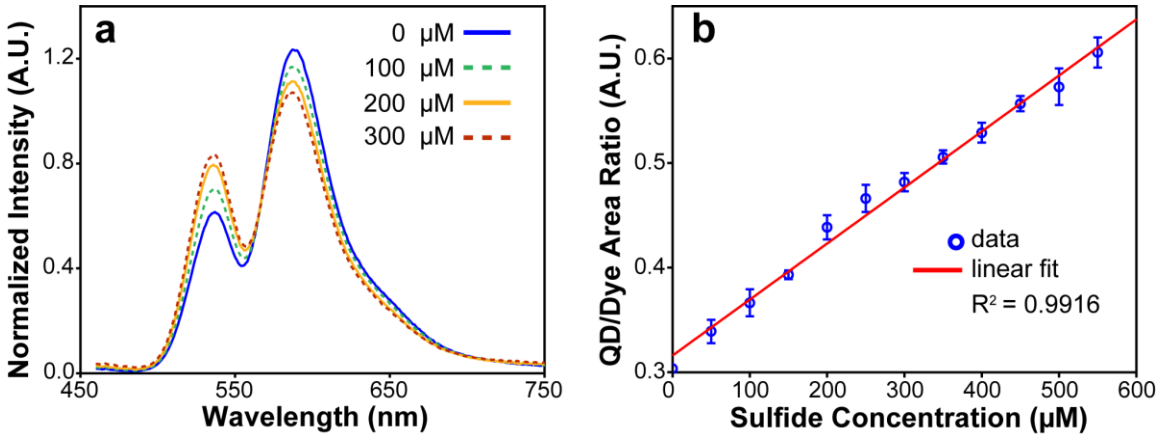
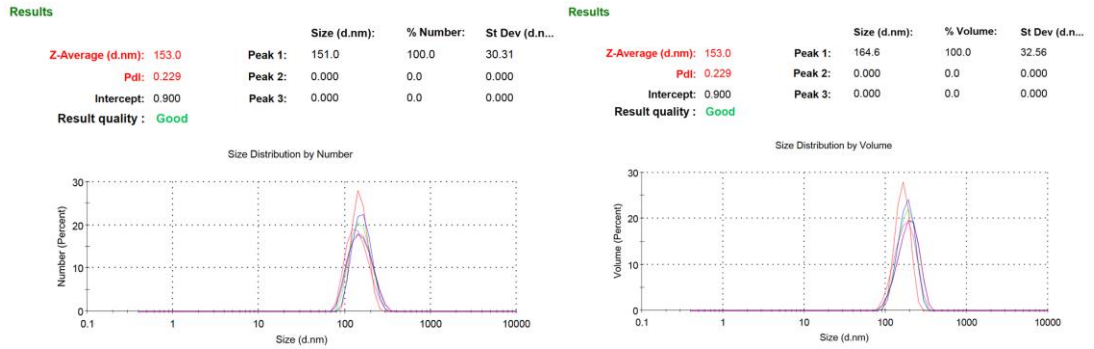


Figure S12. (a) Normalized emission of the PVC modified QD/bisulfide reactive dye sensor (3.4×10^{-8} M) upon exposure to HS^- (raw data appear in Figure S6b). (b) Ratio of the integrated emission of the QD donor over the dye acceptor as a function of HS^- concentration. The detection limit was determined to be 41.9 ± 0.3 μM for a QD sensor concentration of 3.4×10^{-8} M.

PVC Modified QDs:



Uncoated QD:

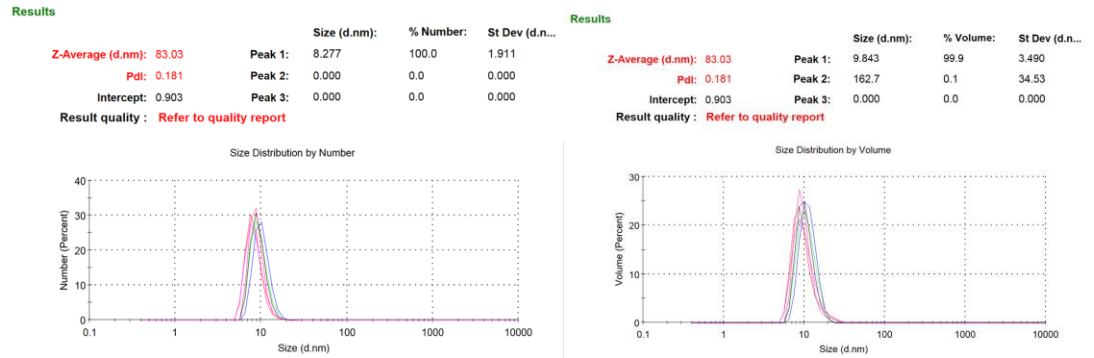


Figure S13. DLS data show that the PVC modified QDs are significantly larger than the unmodified QD samples in water.

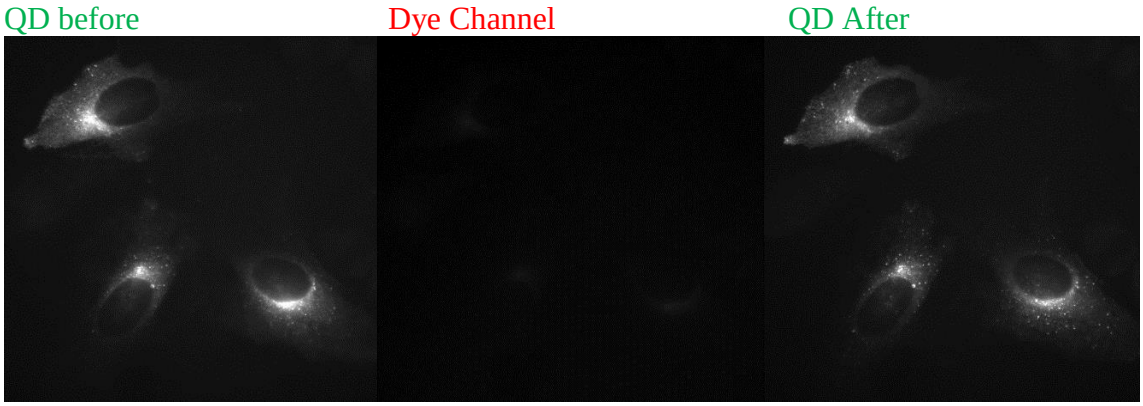


Figure S14. Fluorescence images of water-soluble QDs microinjected into live Hela cells in the QD channel (535 ± 25 nm) before after treatment with Na_2S solution (200 μM) reveal a slight loss of QD intensity. Relatively little QD emission is observed to bleed through the dye channel (610 ± 37.5 nm).

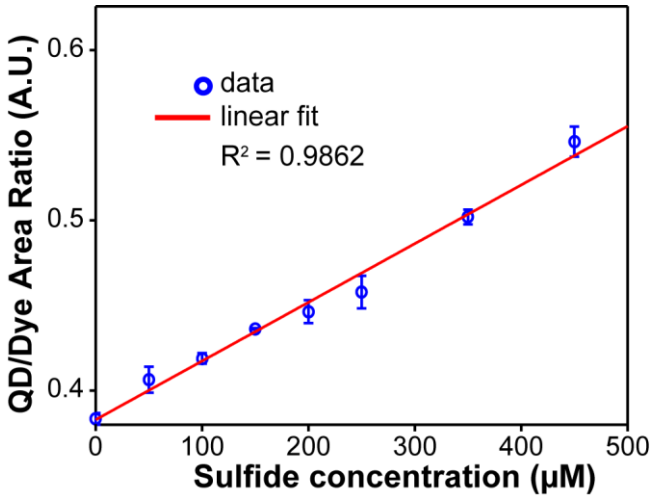


Figure S15. Calibration data from QD/bisulfide-reactive dye conjugates diluted in HS^- standards imaged using the fluorescence microscope, where the ratio is determined from the intensity of the QD channel divided by the intensity of the dye channel.

Cell	Signal Before H_2S Addition	Signal Before H_2S Addition	Signal After H_2S Addition	Signal After H_2S Addition	Change (%)	Change (%)
	QD	**DYE**	**QD**	**DYE**	**QD**	**DYE**
1	1015.884	993.155	1176.631	634.981	**15.8**	**-36.1**
2	1208.503	1194.916	1532.603	811.09	**26.8**	**-32.1**
3	1169.455	1193.748	1304.247	819.378	**11.5**	**-31.4**
4	1073.273	1075.251	1133.617	715.045	**5.6**	**-33.5**
5	1118.225	1182.337	1349.92	860.206	**20.7**	**-27.2**
6	953.788	918.549	1194.263	752.866	**25.2**	**-18.0**
7	866.634	791.696	977.993	556.096	**12.8**	**-29.8**
8	1006.004	987.425	1273.38	728.854	**26.6**	**-26.2**
9	1011.291	953.29	1036.63	643.868	**2.5**	**-32.5**
10	1339.572	1333.454	1695.319	1003.261	**26.6**	**-24.8**
11	1178.619	1141.231	1329.944	880.514	**12.8**	**-22.8**
				Average	**17.0**	**-28.6**
				Error	**±8.7**	**±5.3**

Table S1. QD-Dye signal intensity changes after treatment with Na_2S solution (200 μM).

Cell	Signal Before H_2S Addition	Signal After H_2S Addition	Change (%)
	QD	**QD**	**QD**
1	960.627	852.404	**-11.3**
2	960.56	888.141	**-7.5**
3	956.923	931.068	**-2.7**
4	1160.918	1074.255	**-7.5**
5	1030.104	1003.889	**-2.5**
6	1026.269	940.882	**-8.3**
7	1824.029	1604.722	**-12.0**
8	1098.516	1004.57	**-8.6**
9	1234.09	1109.095	**-10.1**
10	1185.644	928.704	**-21.7**
		Average	**-9.2**
		Error	**± 5.4**

Table S2. Control experiment on water-soluble QDs after treatment with Na_2S solution (200 μM).