Supporting Information for

Synthesis and characterization of DNA-quantum dot conjugates for the fluorescent ratiometric detection of unlabelled DNA

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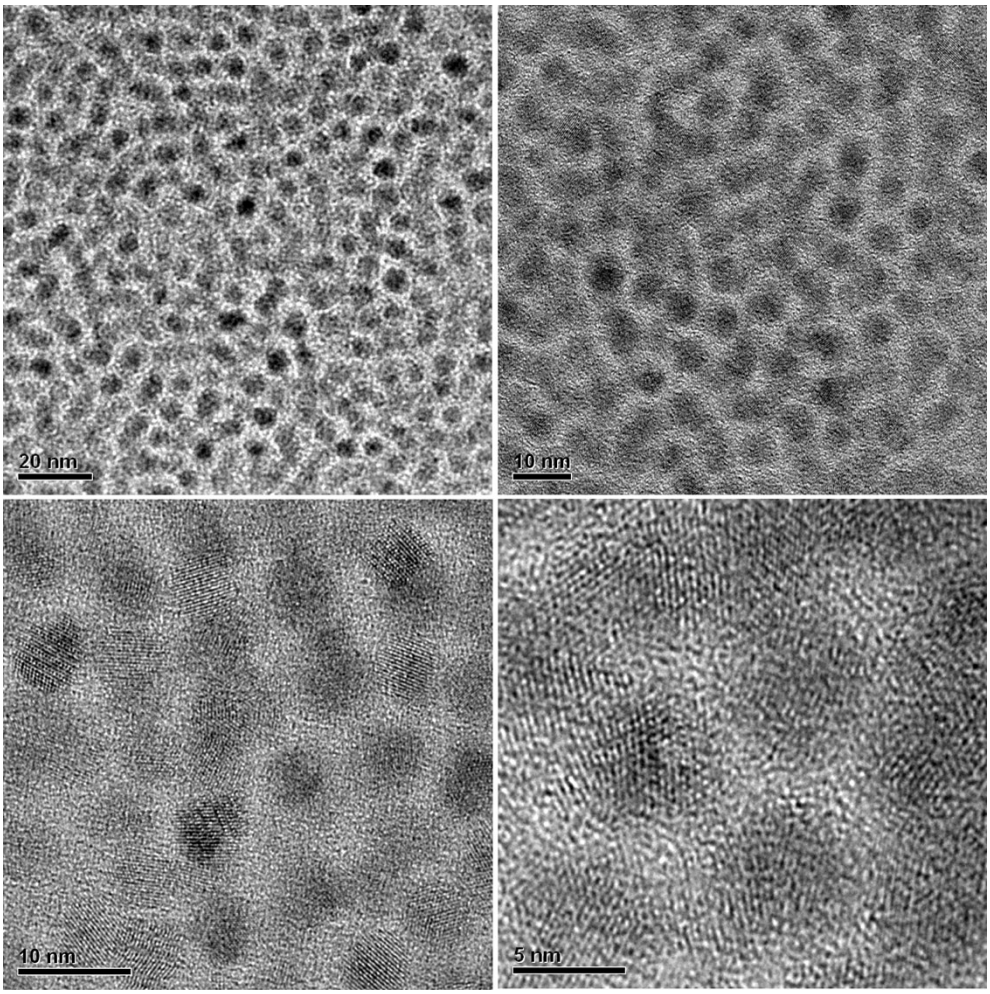

Figure S1. TEM micrographs of 6.38 ± 0.13 nm CdZnS/ZnS QDs reveal uniformity and crystallinity. Note that the hydrodynamic diameter of QDs prepared using cap exchange is on the order of 10 nm.[1]

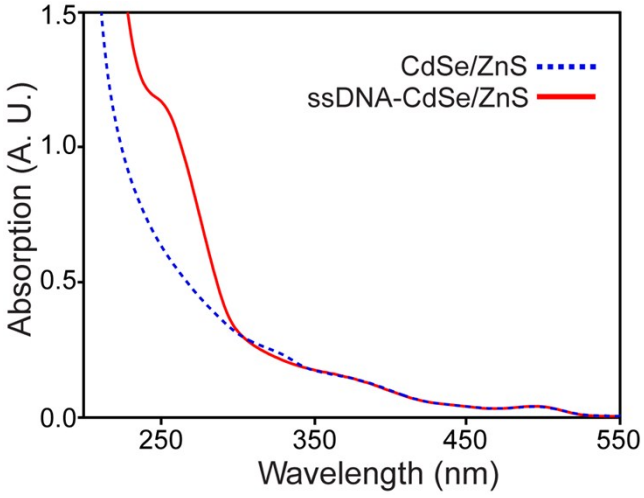

Figure S2. Absorption spectra of water-soluble CdSe/ZnS dots before and after conjugation and dialysis with single-strand DNA (ssDNA) reveal a strong ssDNA feature

at ~260 nm. However, conjugation of longer oligonucleotides (>10 base pairs) proved unsuccessful.

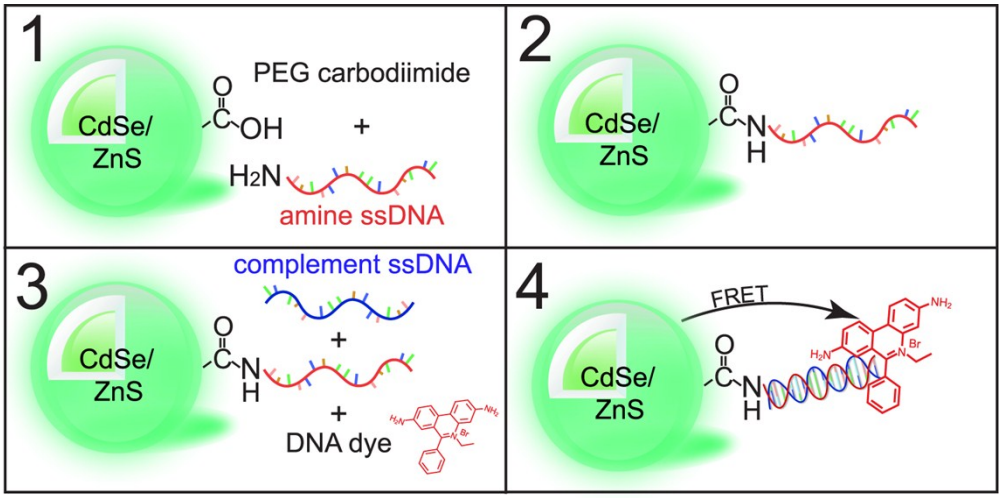

Figure S3. A strategy to create a CdSe/ZnS QD-based DNA sensor. **1, 2.** Conjugation of polymer-coated QDs with amine-functional ssDNA was facilitated using DMTMM or poly(ethylene glycol) carbodiimide.[2, 3] **3, 4.** Exposure to the complementary ssDNA sequence and ethidium bromide dye results in energy transfer from the QD to the DNA intercalating chromophore.

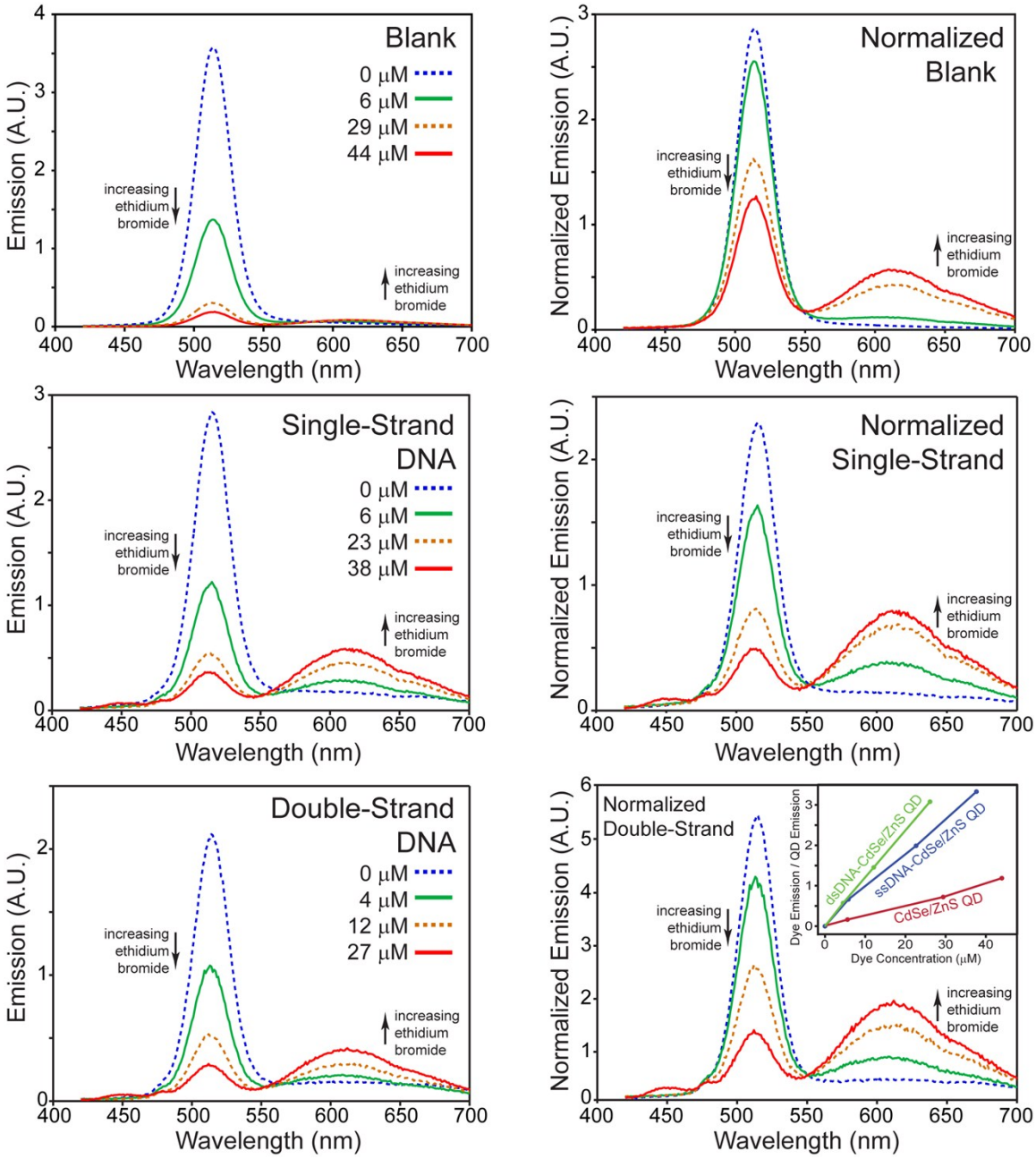

Figure S4. Raw and normalized emission spectra of various samples. Top: Blank CdSe/ZnS QDs, middle: ssDNA-CdSe/ZnS QD conjugates, and bottom: double-stranded DNA (dsDNA)-CdSe/ZnS QD conjugates exposed to increasing levels of ethidium bromide staining dye. The responses of the ssDNA-CdSe/ZnS and dsDNA-CdSe/ZnS conjugates are nearly identical as shown in the inset of the last figure. Normalization is performed with respect to the total area under the emission curve.

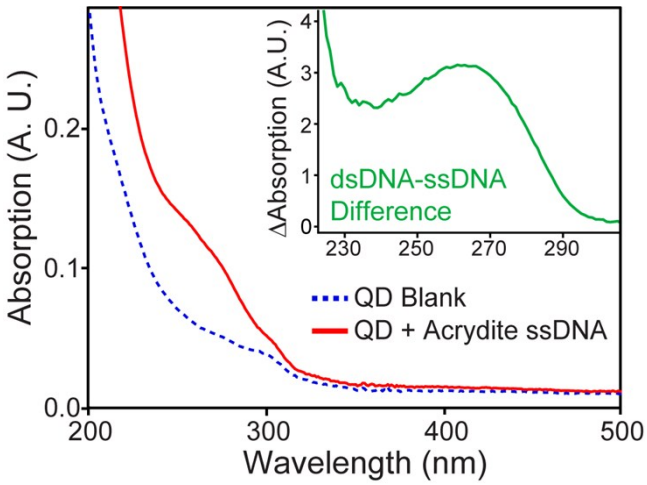

Figure S5. The absorption spectra of silane cap-exchanged CdZnS/ZnS dots before and after conjugation and dialysis with methacrylic phosphoramidite (acrydite)-functional ssDNA reveal strong ssDNA features. Inset shows the difference spectrum after hybridization with the complementary strand to form dsDNA. The change in the absorbance is weak due to the hypochromicity effect.

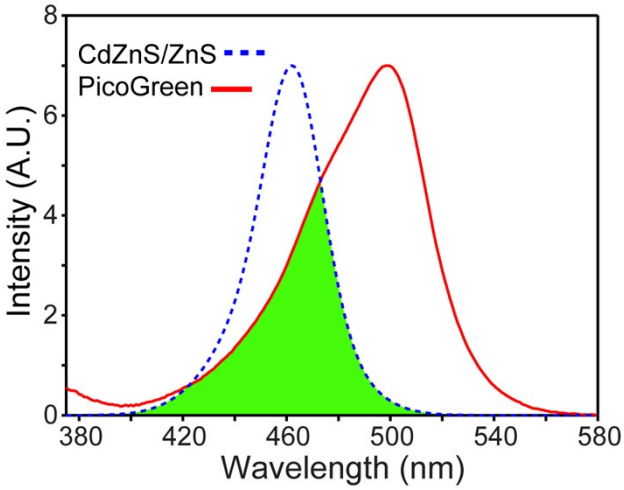

Figure S6. The absorption of Picogreen dye (red solid line) and ssDNA-CdZnS/ZnS QD emission (blue dashed line) demonstrate good spectral overlap to impart efficient FRET efficiency between the QD donor and dye acceptor. The quantum yield of cap exchanged CdZnS/ZnS dots is reported to be 8% in water.[1] PicoGreen extinction at the absorption maximum is 70,000 $cm^{-1}M^{-1}$.[4] These data were analyzed according to the procedures outlined in the supporting information of ref. 5 to reveal a characteristic R_0 of 3.6 nm.

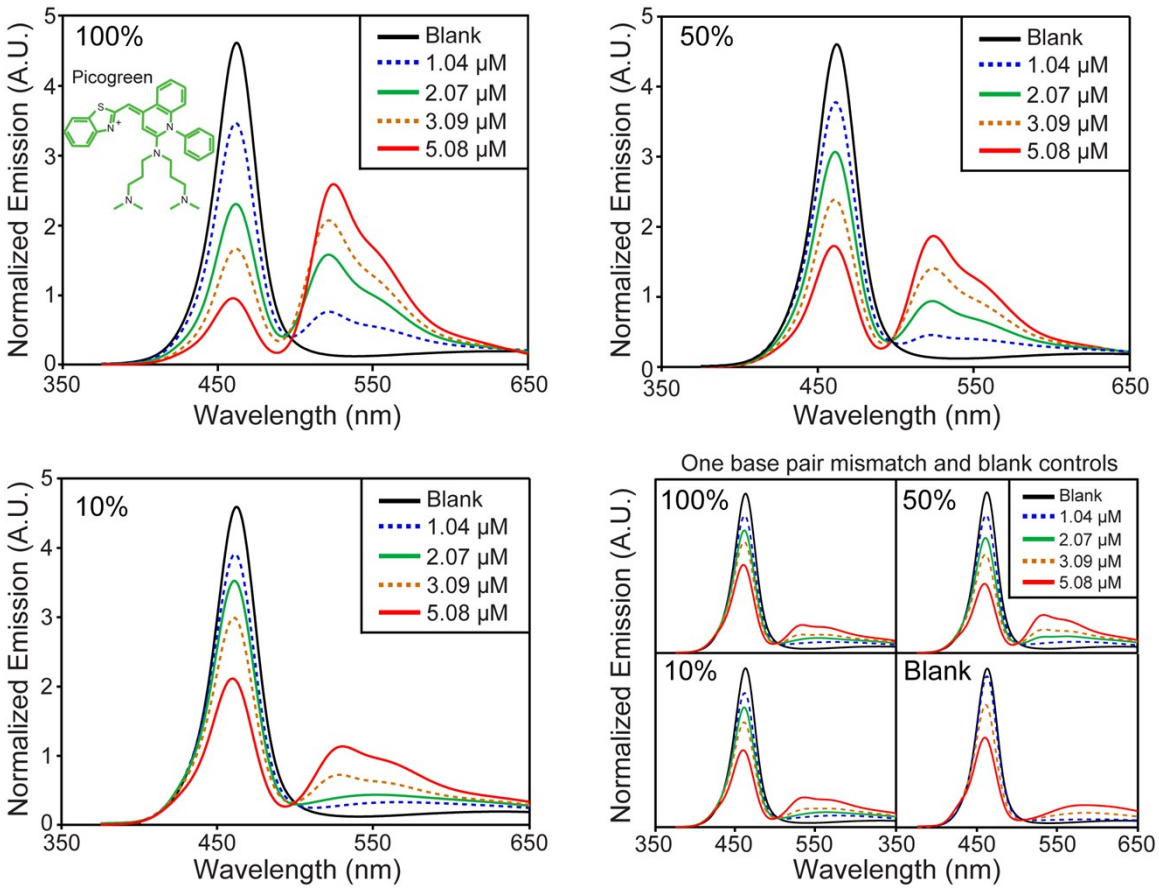

Figure S7. Normalized emission spectra of ssDNA-CdZnS/ZnS QD conjugates as a function of %equivalence exposure to complementary or 1 basepair mismatched ssDNA analytes and titration with PicoGreen. The "Blank" spectra are from bare CdZnS/ZnS dots. Spectra are normalized with respect to the total area under the emission curve.

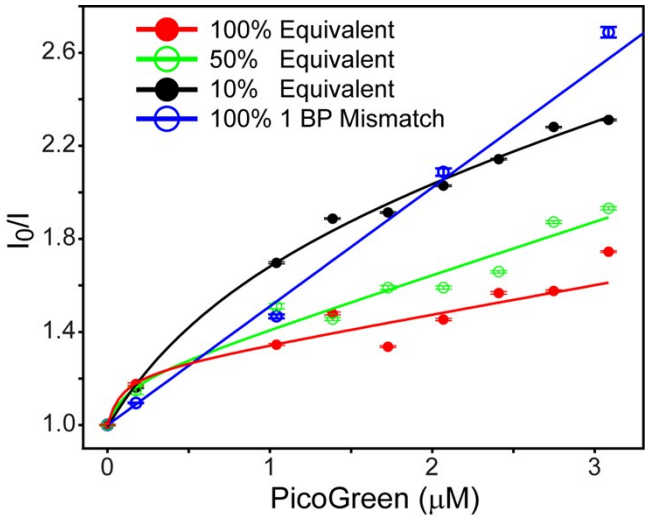

Figure S8. Stern-Volmer plot of dsDNA-CdZnS/ZnS QD quenching due to titration with PicoGreen. The samples were exposed to a %equivalence of ssDNA analytes (complementary and 1 basepair-mismatch) with respect to the probe's ssDNA content.

Sample	C_1	K_{SV} (M^{-1})	C_2	K_{SV} (M^{-1})
Blank QD	1.0	$(8.9\pm1.3)\times10^5$	-	-
100% mismatch	1.0	$(5.10\pm0.13)\times10^5$	-	-
10% equil.‡	0.45±0.15	$(8.4\pm7.9)\times10^4$	0.56±0.14	$(2.1\pm1.2)\times10^6$
50% equil.‡	0.83±0.12	$(1.9\pm0.7)\times10^5$	0.18±0.13	$(1\pm3)\times10^7$
100% equil.‡	0.81±0.11	$(1.0\pm0.6)\times10^5$	0.19±0.13	$(1\pm3)\times10^7$

‡ Integrated emission intensity of quantum dot donors have been fit to:
$\frac{I}{I_o} = \sum_{i=1}^{2} \frac{C_i}{(1 + K_{SVi}[Q])}$, where [Q] is PicoGreen concentration, as outlined in ref. 6. Errors were calculated using the variance-covariance matrix method.

Table S1. Fitted parameters and error to the Stern-Volmer analysis of data presented in Figure S8.

Error Analysis

The proper determination of error can be problematic when analyzing data from high quantum yield ratiometric sensors. This is due to the fact that the S/N ratio of the emission spectra can be quite high and inclusion of all data points in the calculation of the integrated emission ratios results in negligible errors, see for example the error bars in Fig. S8. This is why we didn't include error bars in Fig. 5 of the main text as they appear as lines that cross the center of the data points.

To properly analyze error, we discard the σ of the ratiometric data points and work with the errors of the various fits to the same. We will illustrate the calculation of the limit of detection (LOD) here as an example, and ignore proper significant figure reporting to negate rounding errors. The calculation of the LOD begins with fitting the dye:QD emission ratio of the dsDNA-QD conjugate to the concentration of PicoGreen titrant (Fig. 5B). These data allow us to determine the relationship between the response (i.e. the slope of the line, in units of μM^{-1}) and the %equivalence of analyte exposure (Fig. 5B inset). These data are shown in Table S2. The slope and intercept of this regression was then used to determine how the response of a non-targeted analyte (here, the 100% exposure equivalence of a 1 basepair mismatch sample) would measure up compared to the complementary analyte. While it is tempting to stipulate that this is the LOD, we believe that it is proper to increase the LOD by the standard deviation of the above.

Parameter	Parameter	error
10% equil. slope (M^{-1})	154881.5	18974.96
50% equil. slope (M^{-1})	353064.1	29185.39
100% equil. slope (M^{-1})	895527.3	74006.22
Slope (Fig. 5 inset) (M^{-1})	833676.9	167375.7
Intercept (Fig. 5 inset) (no units)	23196.63	108471.9
100% mismatch slope (M^{-1})	90536.90	11934.02

Table S2. Fitted parameters to the regressions in Fig. 5 used in the calculation of the LOD reported in the main text.

From these data, we can see that the 100% equivalent of the 1 basepair-mismatch sample responds as a %equivalent of the complementary strand of:

$$\frac{90536.9\,\mathrm{M^{-1}} - 23196.63}{833676.9\mathrm{M^{-1}}} = 0.080775$$

or 8.1%.

However, we need to calculate the standard deviation of this result, which is determined using the normal rules for error propagation:

$$0.080775 \cdot \sqrt{\left(\frac{\sqrt{\left(\left(11934.02\mathrm{M^{-1}}\right)^2 + \left(108471.9\right)^2\right)}}{67340.27\,\mathrm{M^{-1}}}\right)^2 + \left(\frac{167375.7\mathrm{M^{-1}}}{833676.9\mathrm{M^{-1}}}\right)^2} = 0.131898$$

or 13.2%

Consequently, we consider that the LOD in the system described herein can be reasonably equated to ~8%→(8+13)% of the number of moles ssDNA recognition element (17.7 nmol) present in each test solution, which corresponds to an absolute number of 1.4→3.8 nmol of ssDNA. As a result, we quote a value of 3.8 nmol in the manuscript.

References

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4. V. L. Singer, L. J. Jones, S. T. Yue and R. P. Haugland, *Anal. Biochem.*, 1997, **249**, 228-238.
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6. M. R. Eftink, L. A. Selvidge, *Biochemistry,* 1982, **21**, 1 17-125.