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COMMUNICATION

pH-Responsive Quantum Dots (RQDs) that Combine a Fluorescent Nanoparticle with a pH- Sensitive Dye

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A quantum dot conjugated to a dye through an experimentally simple process of self-assembly exhibits an enhanced emission when the dye is attached, and this effect is pH-sensitive.

Semiconductor quantum dots (QDs) are inorganic materials with luminescent properties superior to those of conventional organic dyes with respect to photostability, extinction coefficient, broad absorption and narrow photoluminescence (PL) profiles, brightness and fluorescence decay time.[1, 2, 3, 4]

The long decay times exhibited by QDs make them excellent energy donors in Förster Resonance Energy Transfer (FRET)[5] and previous applications of QDs in sensing typically involve FRET from a nanoparticle to an appended dye.[6] To date there have been very few reports of energy transfer in the reverse direction, i.e. excitation of an appended dye that results in energy transfer to the QD.[7, 8, 9, 10] Energy transfer from dye to dot is particularly attractive because it enables selective sensing of analytes *via a chemical process taking place on the dye*, while also taking advantage of the highly desirable *photophysical properties of the nanomaterial*. The QD-dye conjugate reported here behaves in a way that is consistent with reversed energy transfer from dye to dot, and does so in a pH-dependent manner, demonstrating that such a system can indeed be responsive to an analyte. pH-Sensitive QD emission has been achieved in the past by, for example, coupling the QD response to a pH-dependent redox event,[11] or employing synthetically elaborate peptide coatings.[12]

Nanoparticle/dye conjugate QD-ANI was assembled in a straightforward fashion *via* mass-driven ligand exchange with a thiol ligand (Figure 1).[7, 8] Using thiols in this way allows direct labelling on the surface of the dot *via* replacement of capping ligands[13] which is procedurally simpler to alternative approaches involving amide bond formation or metal affinity coordination to the QD surface.[14] The direct thiol approach is further simplified by using the

corresponding disulfide, which gives rise to the thiol *in situ*.[13] (2-(Dimethylamino)ethyl)aminonaphthalimide (ANI) was chosen as the fluorophore for this work, as it is known to be an efficient photoinduced electron transfer (PET) probe for pH.[15] Synthesis of the corresponding disulfide **1** (Figure 1A) was achieved by reaction
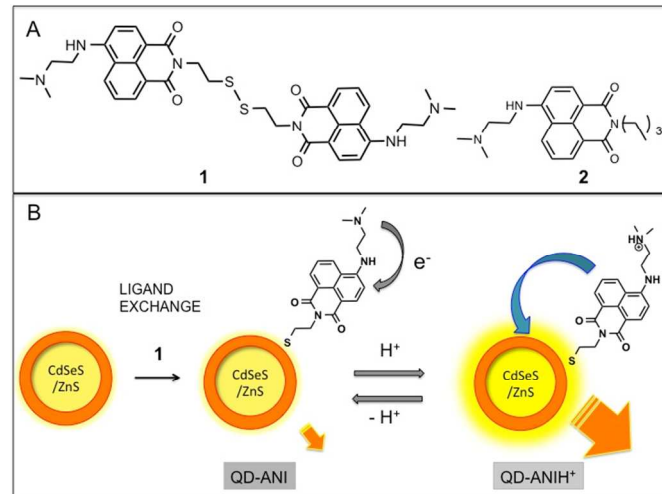

Figure 1: Overview of the responsive QD-ANI system (RQD). **(A)** Ligands investigated in this work and (B) ligand exchange with **1** on Cd/SeS/ZnS core/shell QDs to give conjugate QD-ANI as responsive pH-probe.

of commercially available 4-bromo-1,8-naphthalic anhydride with cystamine under basic conditions followed by amination with a large excess of 2-dimethylaminoethylamine (ESI). The CdSeS/ZnS core/shell type QD with an absorption profile stretching out to 570 nm was chosen since this overlaps well with the emission spectrum of the dye at 535 nm. Initial absorption and emission spectra were

acquired for both the aqueous QD solution in HEPES-buffer (10 mM, pH 7.4, Figure 2a and S5) and disulfide **1** individually (Figure 2b and S1); these data were used as controls against which to reference the effects seen in the spectra of the QD-ANI conjugate. Steady-state fluorescence measurements of the QD in the presence of **1** revealed an increase in QD signal intensity (Figure 2b). The extent of this increase could not be determined directly because of the partial spectral overlap with the ANI emission. However, after deconvolution under the assumption of a linear superposition of **1** and QD emission, an increased QD-emission signal remained (Figure 2b and c): *the QD emission of the QD-ANI conjugate is greater than the simply additive emissions of QD and ANI at this wavelength.* This is potentially due to energy transfer from ANI to QD. Notably, the intensity of the ANI emission is also enhanced, suggesting energy transfer in the reverse direction as well (Figure 2b, green and pink line). (We have separately provided evidence of FRET between QD and dye through conjugation of **1** with a QD with absorption maximum at 450 nm, resulting in the expected decrease in QD-PL and increase in ANI emission).[16] To test the importance of the thiol to the dye/QD interaction the thiol-free dye 2-butyl-6-((2-(dimethylamino)ethyl)amino)naphthalimide (**2**) (Figure 1A) was synthesised and evaluated.[15] Combining **2** and QD under the same conditions showed no increase for both PL signals after deconvolution of the QD PL, thus no energy transfer is observed from the dye **2** to the QD (Figure S12a).

To determine the optimal dye-to-QD ratio, steady-state fluorescence measurements were acquired while titrating increasing amounts of **1** into the QD solution (Figure 2a). Increasing the concentration of **1** up to 1.0 μM brought an increase in PL intensity for both QD and ANI emission. The QD emission maximum remained unchanged at 575 nm throughout the titration while the ANI emission showed a 20 nm blue-shift to 514 nm with respect to the emission of **1**. Spectral shifts of the dye's PL indicative of an interaction with the QD have been reported.[7] At higher concentrations of **1** (>1.0 μM), the emission maximum of the ANI began to shift bathochromically (Figure S6a) and fluorescence enhancement ceased. Equally, the QD emission increase stopped and slightly decreased (Figure 2c, d), suggesting that the surface of the QD was fully covered and additional **1** is not interacting with the nanoparticle's surface. Overall, an increase in QD emission by a factor of 1.27 could be achieved at this concentration ratio, which represents a loading of 40-50 disulfide molecules per one QD (see ESI).[16]
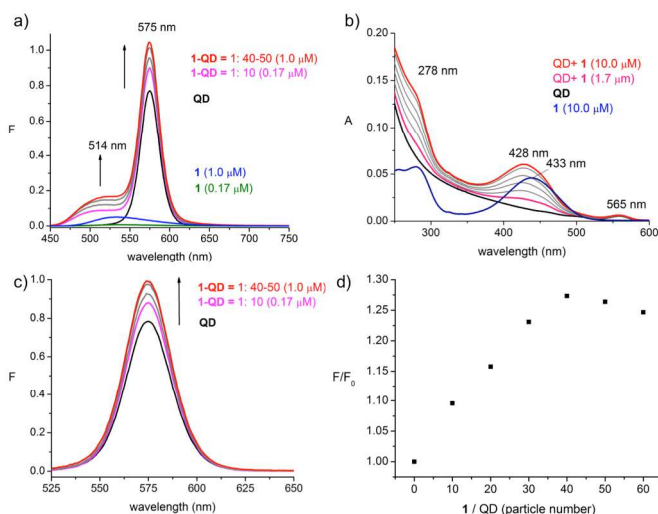

Figure 2: Steady state measurements of dye **1**, the QD and the QD response after mixing with increasing concentrations of **1**: a) UV/Vis absorption spectra of **1** (10.0 μM, blue), QD (0.27 μM, black) and **1**-QD (1.67–10.0 μM, pink to red), b) normalised fluorescence emission spectra of different concentrations of **1** (0.17 μM, green and 1.0 μM, blue), QD (2.67 pM, black) and **1**-QD (0.17–1.0 μM, 1:QD ratio = 10–50, pink to red), c) normalised QD emission response after deconvolution and d) plot of the normalised PL signal change of the QD emission after deconvolution as a function of dye particle to dot ratio.
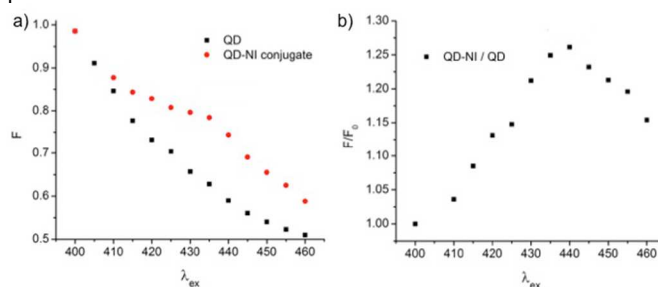

Figure 3: Dependence of excitation wavelength on fluorescence enhancement of QD signal in the QD-ANI conjugate: **a)** normalised PL intensity of QDs (black squares), and of combined QD-ANI signal (after deconvolution, red circles) and b) ratio of QD-ANI intensity over QD intensity at each wavelength.

The same titration was performed at a 10 times higher concentration and was monitored *via* UV/vis spectroscopy (Figure 2b). The absorption spectra showed no indication of changes in the probe's environment or aggregation. However, a slight blue-shift in absorption to 428 nm happens immediately after the first addition of **1** (Figure 2b and S7). Consequently, the PL signal changes of the dye and the QD observed in the titration experiments are solely a result of the specific interaction of dye and dot, rather than aggregation or other macroscopic changes in probe environment.

Attempts were made to isolate the QD-ANI conjugate: at concentrations of **1** below 0.1 μM the conjugate remained soluble and isolation was not possible by centrifugation, while higher concentrations of **1** resulted in a visible QD-ANI pellet of orange colour (Figure S16) due to non-specific interactions of **1** with the carboxylic acid groups of the amphiphilic polymer. Notably, no pellet formed when **2** was combined with QDs (Figure S17).

The influence of the excitation wavelength on the increase of QD fluorescence emission was evaluated. The PL intensity of the QD is naturally enhanced at lower excitation wavelengths due to the intrinsically higher extinction coefficient at these wavelengths (Figure 3a, black squares), so the effect of the dye has to be referenced to the QD signal at the respective excitation wavelength. The normalised PL intensities of the integrated QD emission (black squares) and QD-ANI conjugates (red circles) were determined at different excitation wavelengths 400–460 nm (Figure 3a). The QD-ANI data (red circles) depict clearly deviation from the mono-exponentially progressing dye-free QD. (Note that the acquired QD-ANI spectra were first deconvoluted to show only the modulation of QD emission signal, as described above.) The effective fluorescence enhancement at each wavelength can be determined as the ratio of the intensities of QD-ANI over QD. The resulting graph (Figure 3c) indicates a Gaussian distribution with a maximum at 440 nm, *ca.* 5 nm red-shifted relative to the centre of the lowest-energy absorption band of the dye (435 nm, Figure 2a). (This shift in excitation maximum can be explained by the same electronic effects observed in the emission spectra of the QD-ANI conjugate (Figure 2b), and arises due to the interaction of QD and dye-disulfide.) These results show that the emission intensity of the QD is indeed dependent on the excitation wavelength of the fluorophore, and may indicate energy transfer from the fluorophore in **1** to the QD.
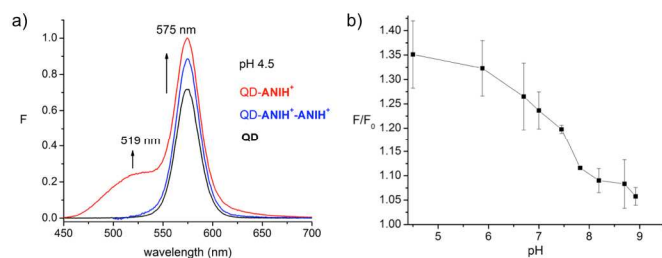

Figure 4: pH-sensitive PL signal changes of the QD emission of the QD-ANI conjugate: a) Normalised fluorescence spectra at pH 4.5 before and after deconvolution, b) Plot of the effective fluorescence enhancement as a function of pH (each data point is the mean of three independent measurements.

To ascertain whether dye/QD assemblies of this type are suitable for sensing applications, the response of the QD-ANI conjugate to solutions of different pH was determined. The constituent parts were first assessed separately for their stability and response over the pH range 4.5–9. While the QD has been reported to be stable over this pH range by the supplier, fluctuations in QD emission intensity were nonetheless observed (Figure S8). Disulfide **1** showed the expected decrease in fluorescence emission with increasing pH[15] and a sigmoidal relationship between integrated intensity and pH (Figure S2). The calibration curve differs in shape from that recorded for the reference compound **2** (Figure S4) and those reported previously for related compounds,[15] especially at lower pH; this is presumably due to the effects of excimer formation seen with bis-naphthalimide dyes connected by short linkers.[17, 18]

Steady state fluorescence measurements of the QD-ANI conjugate in solutions of different pH clearly showed that the QD emission was higher at low pH (Figure 4a), reducing in signal intensity as the pH was increased (Figure 4b and S10). The individual PL spectra of the QD-ANI conjugate at each value of pH were referenced to the QD emission at the same pH to calculate the effective fluorescence enhancement (Figure S9); the effective fluorescence enhancement is *ca.* 1.35 at pH 4.5 but only 1.05 at pH 9.0. The absorbance was monitored at three distinct pH values over 1 hour and did not show any suggestion of aggregation (Figure S15).

This plot of fluorescence enhancement factors *vs.* pH reveals the same sigmoidal relationship and pKa (*ca.* 7.5) as found for the dye alone (ESI). Control experiments with reference compound **2** at different values of pH did not show any enhancement of the QD emission, while the fluorophore emission showed the expected pH-dependent response (Figure S11-14). These results confirm that in the QD-ANI conjugate, the fluorescence output of the QD is not only enhanced in the presence of the dye, but that this enhancement is responsive to changes in the dye's fluorescent properties. Thus protonating the dimethylamino group interrupts PET in the dye, leading to greater fluorescence enhancement in the QD-ANI conjugate.

In summary, we have shown that the dye-disulfide **1** can be readily assembled onto the surface of a Cd/SeS/ZnS QD, and that the resulting conjugate functions as a probe for pH. While the effective fluorescence enhancement of the QD is low we were able to show that interruption of PET in the pH-sensitive dye modulates the signal response of the QD intensity. This responsive QD (RQD) strategy may now be used to develop conditions that afford greater signal amplification, and extend the principle to create QD probes that are responsive to other analytes.

Notes and references

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