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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.[1–4]

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.[7–10] 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 (Fig. 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** (Fig. 1A) was achieved by reaction 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, Fig. 2a and Fig. S5, ESI†) and disulfide **1** individually (Fig. 2b and Fig. S1, ESI†); 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**

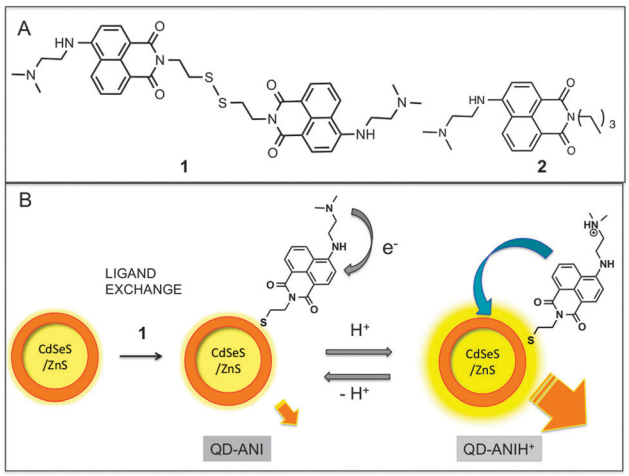

Fig. 1 Overview of the responsive QD–ANI system (RQD). (A) Ligands investigated in this work and (B) ligand exchange with **1** on Cd–SeS–ZnS core–shell QDs to give conjugate QD–ANI as responsive pH-probe.

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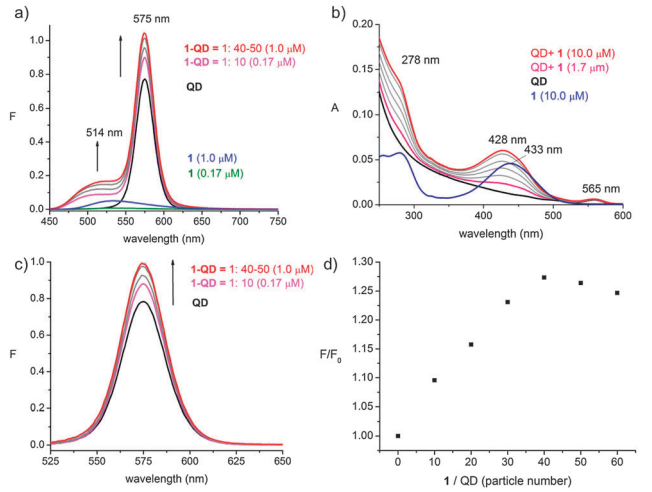

Fig. 2 Steady state measurements of dye **1**, the QD and the QD response after mixing with increasing concentrations of **1**: (a) normalised fluorescence emission spectra of different concentrations of **1** (0.17 μM, green and 1.0 μM, blue), QD (2.67 pM, black) and **1**-QD (0.17–1.0 μM, **1** : QD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

revealed an increase in QD signal intensity (Fig. 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 (Fig. 2a, c and d): 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 (Fig. 2a, 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**) (Fig. 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 (Fig. S12a, ESI†).

To determine the optimal dye-to-QD ratio, steady-state fluorescence measurements were acquired while titrating increasing amounts of **1** into the QD solution (Fig. 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 (Fig. S6a, ESI†) and fluorescence enhancement ceased. Equally, the QD emission increase stopped and slightly decreased (Fig. 2c and 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]

The same titration was performed at a 10 times higher concentration and was monitored *via* UV/vis spectroscopy (Fig. 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** (Fig. 2b and Fig. S7, ESI†). 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 (Fig. S16, ESI†) 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 (Fig. S17, ESI†).

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 (Fig. 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 (Fig. 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 (Fig. 3b) 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, Fig. 2a). (This shift in excitation maximum can be explained by the same electronic effects observed in the emission spectra of the QD–ANI conjugate (Fig. 2a), and arises due to the interaction of QD and dye-disulfide.)

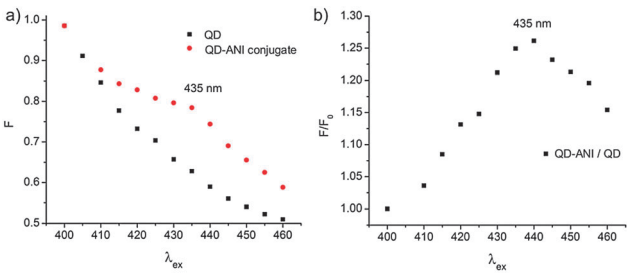

Fig. 3 Dependence of excitation wavelength on fluorescence enhancement of QD signal in the QD–ANI conjugate: (a) integrated PL intensity of QDs (black squares), and of combined QD–ANI signal (after deconvolution, red circles) and (b) ratio of QD–ANI intensity over QD intensity at each wavelength.

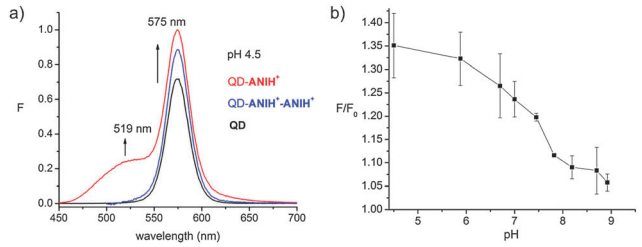

Fig. 4 pH-Sensitive PL signal changes of the QD emission of the QD–ANI conjugate: (a) normalised fluorescence spectra at pH 4.5 before and after deconvolution, (b) plot of the effective fluorescence enhancement as a function of pH (each data point is the mean of three independent measurements).

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.

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 (Fig. S8, ESI†). Disulfide **1** showed the expected decrease in fluorescence emission with increasing pH[15] and a sigmoidal relationship between integrated intensity and pH (Fig. S2, ESI†). The calibration curve differs in shape from that recorded for the reference compound **2** (Fig. S4, ESI†) 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 (Fig. 4a), reducing in signal intensity as the pH was increased (Fig. 4b and Fig. S10, ESI†). 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 (Fig. S9, ESI†); 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 (Fig. S15, ESI†).

This plot of fluorescence enhancement factors *vs.* pH reveals the same sigmoidal relationship and pK_a (*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 (Fig. S11–S14, ESI†). 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.

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