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ORIGINAL PAPER

Anion Sensing with Luminescent Quantum Dots – A Modular Approach Based on the Photoinduced Electron Transfer (PET) Mechanism

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Abstract A CdSe–ZnS quantum dot (QD) has been surface functionalised with 1-(2-mercapto-ethyl)-3-phenyl-thiourea in the fluorophore–spacer–receptor format typical of Photoinduced Electron Transfer (PET) based organic dye sensors. The resulting QD conjugate was tested for selectivity toward the tetrabutylammonium salts of fluoride, chloride, bromide, hydrogen sulfate and acetate. Addition of fluoride, chloride and acetate ions resulted in an approximate 90% quenching of the original fluorescence intensity, while bromide and hydrogen sulfate had almost no effect. The observed quench was attributed to an increase in the reduction potential of the receptor upon anion binding resulting in an increase in PET from the excited QD to the receptor and a concomitant reduction in fluorescence intensity. The selectivity and sensitivity were comparable to an analagous organic dye based sensor where a similar receptor was bound to an anthracene fluorophore. Thus a modular approach is evident where a receptor used in an organic dye based sensor can be adapted and successfully used with QD's.

Keywords Quantum dots · Photoinduced electron transfer · Thiourea · Anions · Luminescent · Sensor

Introduction

Since their discovery in the early 1980s, luminescent Quantum Dots (QDs) have rapidly emerged as viable alternatives to organic dyes for use in bio-labelling applications [1–3]. Their high quantum yields, reduced susceptibility to photobleaching, broad absorption spectra and size-dependent emission spectra make them ideal for use in this type of environment. However, their development as the signalling unit in luminescent sensors has not been as rapid. Among the current approaches to sensing with QDs, Forster Resonance Energy Transfer (FRET) based systems have found greatest success [4–7]. Although examples do exist of QD sensors that operate using an electron transfer mechanism, these are primarily for re-dox active transition metals [8–13], involve elaborate conformational manipulations in their design [14, 15] or are two component systems where a quencher is first added to a solution of the QD followed by the addition of an analyte [16]. Quite recently though, Raymo and co-workers have demonstrated a pH dependent "Off – On" switching QD – oxazine conjugate, where modulation of the oxidation potential of the oxazine was shown to be responsible for the luminescent switching behaviour of the QD [17]. Inspired by this we have designed a QD based sensor for anions according to the Photoinduced Electron Transfer (PET) design principle. PET based sensing is well established with organic dyes and proves popular as its modular nature offers a degree of simplicity and predictability [18–26]. The application of this design principle to QDs would provide the opportunity for many more QD based probes, thereby taking advantage of their superior optical properties. We have adopted the Fluorophore-Spacer-Receptor format of PET systems to generate the first reported example of a PET operated QD probe for anions (Fig. 1). Gunnlaugsson *et al* have previously shown that charge neutral thiourea's were useful receptors for anions when bound to an anthracene fluorophore in a PET format [27]. Therefore we selected 1-(2-mercapto-ethyl)-3-phenyl-thiourea as receptor and bound it to the surface of a

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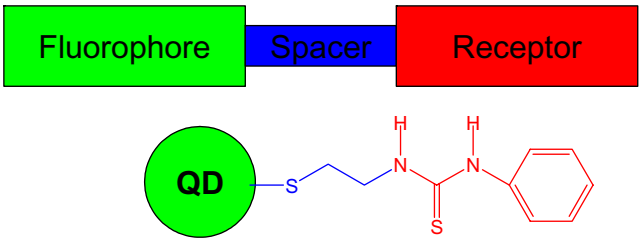


Fig. 1 Schematic representation of the fluorophore – spacer – receptor format of PET systems (*top*) and the analogous QD – spacer – thiourea receptor conjugate used in the present study

green emitting CdSe–ZnS QD. The resulting QD-spacer-receptor conjugate was tested against the tetrabutylammonium salts of fluoride, chloride, bromide, hydrogen sulfate and acetate and the selectivity and sensitivity compared against its organic counterpart.

Experimental

Reagents and materials

All reagents were purchased from Aldrich at the highest quality available. CdSe–ZnS QDs were purchased from Evident technologies, New York (Product No. ED-C10-TOL-0545).

UV and fluorescence spectroscopy

Absorbance measurements were recorded on an Agilent UV–Vis Spectrometer using 10 mm quartz cuvettes. Fluorescence measurements were recorded on a Perkin Elmer LS55 Luminescence Spectrometer using 10 mm quartz cuvettes. Excitation wavelength unless otherwise stated was set at 370 nm. Excitation slit size was 10.0 nm and emission slit size was 10.0 nm. Scan speed was set at 500.

^{1}H and ^{13}C-NMR spectroscopy

All spectra were recorded on a Bruker Ultrasheild 400 MHz. ^{1}H NMR samples were prepared by dissolving 5 mg of sample in 1.0 mL of $CDCl_3$. ^{13}C NMR samples were prepared by dissolving 50 mg of the sample in 1 mL of $CDCl_3$. Chemical shifts are reported in parts per million (δ) downfield of TMS.

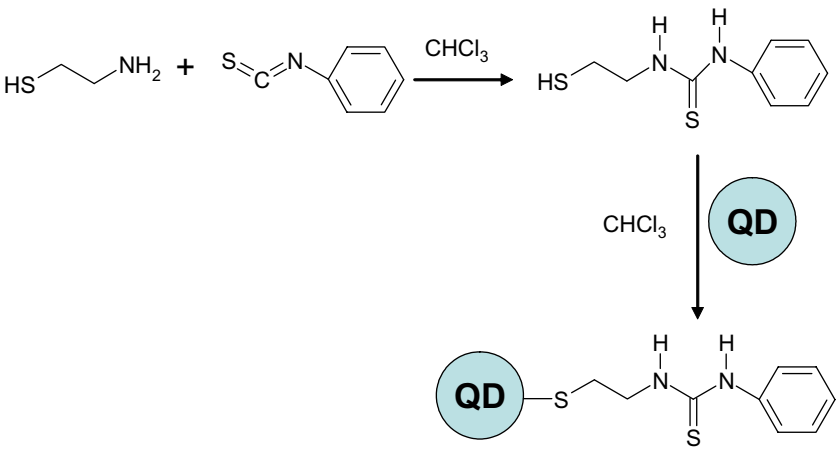
Synthesis of 1-(2-mercapto-ethyl)-3-phenyl-thiourea

To a solution of 2-aminoethanethiol (2.0 g, 26 mmol, 1 eq) in anhydrous chloroform (25 mL) was added phenyl isothiocyanate (3.13 mL, 26.3 mmol, 1.eq). The contents were stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure yielding a white solid. This was triturated with diethyl ether (20 mL) and ethanol (50 mL) was added to the precipitate and the contents stirred for 30 min. The product was filtered under reduced pressure and washed with ethanol (50 mL) and dried in vacuo. The crude product was purified by dissolving in a hot solution of methanol/ethyl acetate and precipitating with cold hexane. Yield=2.49 g, 45.2%. ^{1}H NMR ($CDCl_3$) δ=7.66 (br.s, 1H, PhN*H*), 7.43 (dd, *J*=7.8 Hz, 4 Hz, 2H, aryl), 7.33 (dd, *J*=7.8 Hz, 4 Hz, 1H, aryl), 7.31 – 7.00 (m, 2H, aryl), 6.49 (br.s, 1H, $-CH_2NH$), 3.96 (q, *J*=4.0 Hz, 2H, $-SCH_2-$), 2.95 (t, *J*=6.0 Hz, 2H, $HNCH_2$). ^{13}C NMR ($CDCl_3$) δ, 180.91 (C, −C=S), 135.85 (CH), 130.28 (CH), 127.61 (C), 125.41 (CH), 43.85 (CH_2), 37.14 (CH_2). ESMS Expected: m/z 213 (M^+ + H), 445 (Disulfide + Na^+); Found: 445 (100%), 213 (10%), 211 (70%). Melting point=150 °C.

Ligand exchange reaction

The procedure developed by Tomasulo et al. was followed for ligand exchange [17]. A solution of CdSe/ZnS core

Scheme 1 Synthesis of QD-spacer receptor conjugate



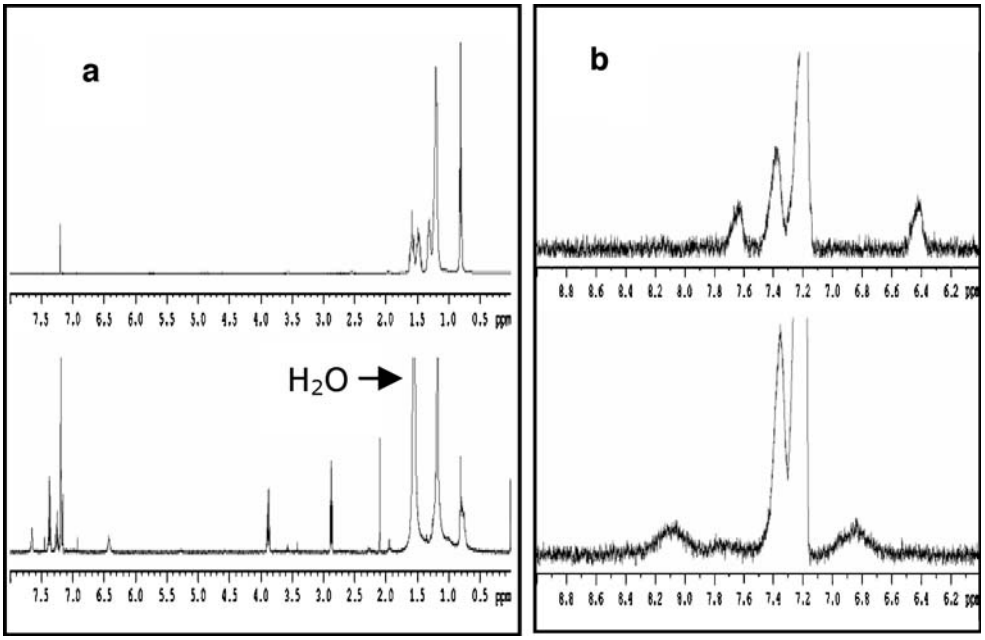


Fig. 2 **a** showing ^{1}H nmr spectra of the free QD (*top*), and the QD-ligand conjugate (*bottom*). **b** expansion of the receptor region showing free receptor (*top*) and after the addition of 1 eq of AcO^{-} (*bottom*). Both spectra recorded in $CDCl_3$ at 400 MHZ

shell QDs (0.5 mL, 0.027 mol) and 1-(2-mercapto-ethyl)-3-phenyl-thiourea (0.03 g, 150 mol) in chloroform (20 mL) was heated under reflux for 24 h. After cooling to ambient temperature, the solvent was removed under reduced pressure. The residue was suspended in acetonitrile (8 mL) and centrifuged at 12,500 rpm for 5 min. The centrifugation step was repeated three more times to afford the modified CdSe/ZnS core shell QDs as a yellow powder (0.015 g).

Results and discussion

The receptor, 1-(2-mercapto-ethyl)-3-phenyl-thiourea was synthesized in one step by reaction of phenylisothiocyanate and 2-aminoethanethiol (Scheme 1). The thiol terminated ethyl spacer permits anchorage to the nanoparticle fluorophore after refluxing in chloroform for 24 h. The nanoparticle chosen was a 2.4 nm CdSe/ZnS QD purchased from Evident Technologies [28]. The resulting QD-Spacer-Receptor conjugate was isolated by precipitation with acetonitrile [17].

Surface functionalisation was confirmed by ^{1}H nmr spectroscopy, Fig. 2a showing spectra of the free QD and the QD-Receptor conjugate. As observed in a previous study, the addition of the receptor does not displace all the ligands on the surface of the QD with shifts at 0.8 and 1.2 ppm representing the methyl and methylene protons respectively of trioctylphosphine (TOP), a strongly coordinating surfactant used in the synthesis of the core QD [4]. Clearly evident though are the urea protons, CH_2-N*H* and Ar-N*H* at 6.4 and 7.7 ppm, respectively, with the signals at 2.9 and 3.9 ppm representing the methylene protons for N-CH_2 and S-CH_2 respectively of the spacer. UV–Vis analysis before and after surface functionalization (Fig. 3) shows no difference in the visible region, the position of the

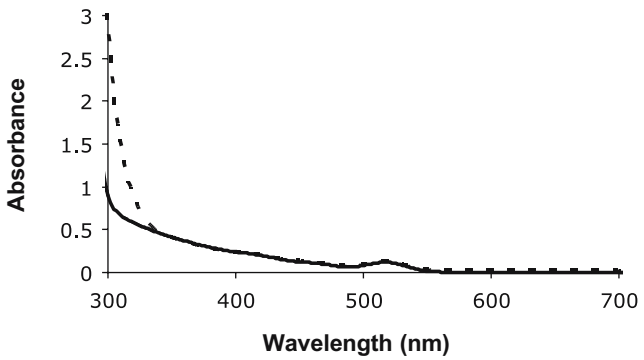


Fig. 3 UV trace of QD before (*solid line*) and after (*dashed line*) surface functionalisation with thiourea recorded in $CHCl_3$. Concentration = 1.81×10^{-6} M

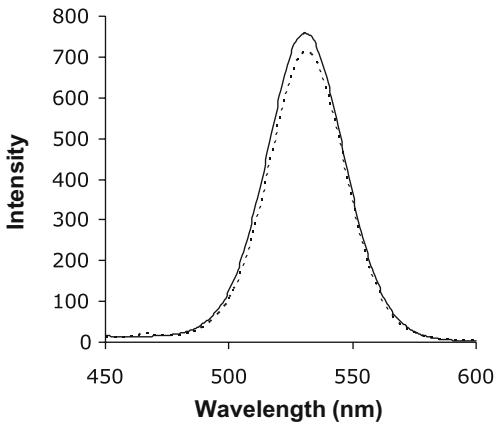


Fig. 4 Fluorescence Spectra of QD before (*dashed line*) and after (*solid line*) ligand exchange with 1-(2-mercapto-ethyl)-3-phenyl-thiourea

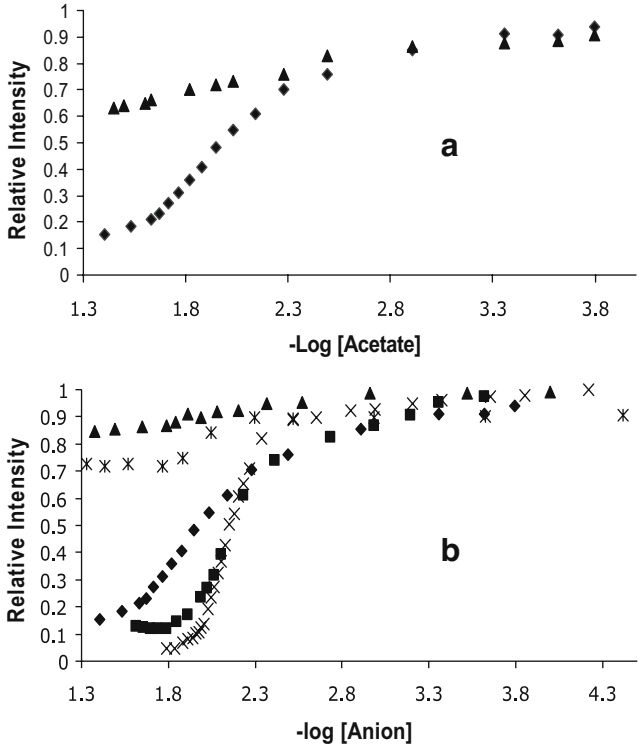


Fig. 5 **a** Plot of relative intensity against – log [acetate] for as received QDs (*filled triangle*) and the QD-receptor conjugate (*filled diamond*). [QD]=5.45×10^{-9}, [QD-Receptor]=7.96×10^{-9}; **b** plot of relative intensity against −log [anion] for Br^- (*filled triangle*), HSO_4^- (*), Cl^- (X), AcO^- (*filled diamond*) and F^- (*filled square*). [QD–Receptor]=7.96×10^{-9} M. All measurements conducted in $CHCl_3$

first exciton peak being unchanged at 516 nm. When the optical density was fixed at 0.1 for both samples at this wavelength a difference was observed at ~ 300 nm with the absorbance of the QD-receptor conjugate greater (2.9) than the QD alone (1.1). The increase in absorbance at this wavelength is due to absorbance by the phenyl choromophore (λ_{max}=264) of the receptor component.

Excitation of the QD-spacer–receptor conjugate at 370 nm resulted in emission at 540 nm, with little change in the profile compared to the parent QD (see Fig. 4), although the quantum yield (Φ) did drop from 0.38 to 0.33. This suggests a small degree of PET quenching even in the absence of anions [27, 29].

Table 1 Photophysical properties for sensor before and after the addition of anions

Sensor	Φ_F^a	$\%F_{red}^b$	Log β^c
Free sensor	0.334	–	–
F^-	0.042	87.3	2.15
Cl^-	0.015	95.4	2.15
Br^-	0.281	15.7	–
AcO^-	0.037	88.9	1.94
HSO_4^-	0.268	19.8	2.12

[a] Quantum yield calculated with reference to fluorescein
[b] % reduction in fluorescence spectral area
[c] Binding constant calculated from a plot of $-\log (F_{MAX}-F)/(F_{MIN}-F)$ against −log [onion]

The probe was then screened for selectivity against the tetrabutylammonium salts of AcO^-, F^-, Cl^-, Br^- and HSO_4^- in chloroform. A plot of relative intensity against −log [anion] is shown in Fig. 5b and reveals significant quenches for Cl^-, F^- and AcO^- with only minor changes observed for Br^- and HSO_4^-. In contrast, addition of these anions to a solution of the "as purchased" QDs (i.e. with no thiourea receptor attached) produced only minimal quenching (shown for AcO^- in Fig. 5a). This suggests anion binding to the thiourea receptor must be responsible for the modulation of the fluorescence output. To investigate this further we recorded the ^{1}H NMR spectrum of the QD – receptor upon addition of 1 equivalence of AcO^-. Anion-receptor binding was confirmed by changes in the chemical shift of the urea protons, with CH_2-N*H* moving downfield by 0.4 ppm to 6.8 ppm and Ar-N*H* by 0.5 to 8.1 ppm upon addition of 1 eq of AcO^- (Fig. 1b). In addition, these resonances become significantly broader after anion addition while the aromatic protons and the methylene protons of the spacer (not shown) remain unaffected. This data suggests a hydrogen bonding interaction between the acetate anion and thiourea protons of the receptor as depicted in Fig. 6a. In terms of a previously described model, the quenching effect by Cl^-, F^-, and AcO^- is a result of this hydrogen bonding interaction and leads to an increase in the reduction potential of the receptor, enhancing the rate of PET from the HOMO of the receptor to the QD [27, 30, 31]. This is

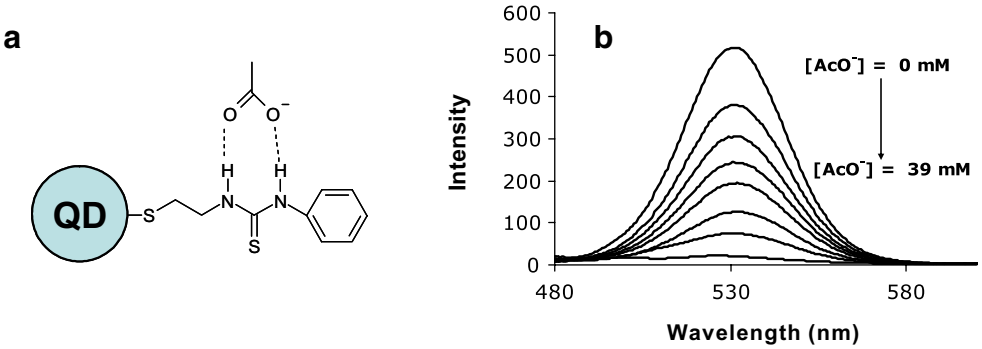


Fig. 6 **a** Schematic representation of QD-spacer-receptor on addition of acetate ion and **b** fluorescence spectra of QD-conjugate upon addition of increasing amounts of Acetate ion

opposite to typical PET sensing behavior where the oxidation potential of the receptor is raised upon analyte recognition, thus removing the thermodynamic driving force for PET and fluorescence is "Switched On" [17].

From Fig. 5b it can also be observed that acetate anions quench fluorescence less efficiently than fluoride or chloride over about 2 log units signifying a 1:1 binding [27]. The selectivity of the receptor for F^- and Cl^- over Br^- can be related the higher charge density of these ions enabling them to form strong hydrogen bonds with the receptor, although steric reasons may also play a part. Steric factors may also explain the selectivity for acetate over hydrogen sulfate, with the former able to form stronger linear hydrogen bonds [27].

The binding constants, Log β were calculated from equation 1 [32, 33] and the values are presented in Table 1.

$$-\log\left(F_{MAX}-F\right)/\left(F-F_{MIN}\right)=\mathrm{Log}[\mathrm{Anion}]+\mathrm{Log}\,\beta \quad (1)$$

where F_{MAX} is the maximum fluorescence intensity, F_{MIN} is the minimum fluorescence intensity and F the observed fluorescence intensity. The values compare favorably with those observed by Gunnlaugsson *et al* in their study into the ability of this ligand to sense anions when connected in a PET format to anthracene [27]. For example we find log β for AcO^- and F^- to be 1.94 and 2.15, respectively, compared to 2.15 and 2.90, respectively, found by them. The slight reduction in sensitivity observed by us may be due to an extra methylene unit in the spacer (which was included for synthetic convenience) reducing the effectiveness of PET, as PET efficiency has previously been shown to be distance dependent [34].

Conclusion

We have demonstrated for the first time that a receptor component from a PET based organic dye sensor can be adapted and used effectively with Quantum Dots. Thus, a modular approach is evident in which a tried and tested receptor from the literature can be modified slightly and used to produce a functioning QD sensor. Moreover, this approach should ensure that the selectivity and sensitivity of the receptor is retained but the enhanced optical properties offered by the QDs are utilized. We believe this approach could lead to a new generation of luminescent sensors.

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