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Luminescent Sensing with Quantum Dots

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Summary

This review highlights recent advances in the use of quantum dots (QD's) as luminescent sensors. The bulk of the study concentrates on systems that possess organic ligands bound to the surface of QD's. These ligands vary from low molecular weight thiols to larger molecules such as maltose binding protein. All have one thing in common: when a target analyte binds to the ligand/receptor, a perturbation of the system occurs, that registers itself as a change in the luminescence intensity of the QD. Two main mechanisms are prevalent in controlling the luminescent intensity in such systems. The first is Photoinduced Electron Transfer (PET) and the second energy transfer. This review looks at current sensors that operate by using these mechanisms. Two component systems are also investigated where a quencher is first added to a solution of the QD, followed by addition of the target analyte that interacts with the quencher to influence the luminescence intensity.

Introduction

Semiconducting nanocrystals, otherwise known as Quantum Dots (QD's) were first discovered in the early 1980's [1]. Since then, interest in QD's as alternatives to traditional organic dyes has increased dramatically. Their use has mainly been restricted to fluorescent labelling in biological environments [2–4], but recently more and more examples of QD based sensor systems can be found in the literature. The superior optical properties of QD's compared to organic dyes is the main attraction to the user. These include broad absorption spectra, higher quantum yields, reduced photobleaching, and narrow emission spectra without red tailing [3]. But the most endearing feature must be the size dependent nature of the emission wavelength. By controlling the growth of the nanocrystal the emission wavelength can be tailored. This, in addition to their broad absorption spectra means QD's of different sizes can all be excited with a single wavelength. The possible applications for such systems are many.

This review will be restricted to those examples where QD's have been incorporated into sensor systems. Primarily, the focus will be on those QD's with bound organic ligands and the selectivity they display towards metal ions and small organic molecules. The application of QD's as biolabels, and those sensors using phosphorescent emission will not be studied here but are covered elsewhere [5].

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Synthesis of QD's

Core QD synthesis

Typically, QD's consist of a group IIB metal, usually cadmium, combined with a chalcogenic element e.g. CdS, CdSe or CdTe [6]. The synthetic preparation normally involves the high temperature addition of a Cd^{2+} source ($CdMe_2$ or CdO) to a chalcogenic element (S, Se or Te) in a strongly co-ordinating solvent such as trioctylphosphine (TOP) or trioctylphosphine oxide (TOPO) [4]. The reaction time, temperature and metal to chalcogenide ratio can be varied to control the size of the nanocrystal and hence its spectral properties.

The pioneering work of Peng [7] in using CdO as an alternative to the highly toxic and pyrophoric $CdMe_2$ has no doubt been responsible for the increased willingness of scientists to synthesise QD's. The quality of the nanocrystals produced from this much easier and safer synthetic approach are comparable to those obtained from $CdMe_2$. In fact, QD's of various size's can now be prepared in undergraduate laboratories with simple apparatus in less than 1 h [8].

Core-shell synthesis

Core QD's usually require surface capping to produce materials with high quantum efficiencies. The reasons for surface capping are mainly to prevent aggregation of the QD's caused by steric hindrance or charge and to

passivate dangling bonds at the surface. Surface passivation involves coating the core QD with a substance that has a larger band gap such as ZnS. Modified thiols can also be used to cap the core and also provide an opportunity for structural modifications [9].

Size selective precipitation

Core and core-shell QD's are purified/isolated by centrifugation. First, the addition of a non-solvent to a colloidal suspension of the QD is required to induce flocculation. The flocculated suspension is then centrifuged and the nanocrystal collected as the solid. This procedure is repeated if necessary.

Ligand exchange

In order to tailor the physical properties of QD's to their desired use i.e. as biolabels or sensors, the surface ligands (TOP/TOPO) must be exchanged with ligands of suitable functionality. Surface ligand displacement normally occurs by heating a solution of the desired ligand with the Core QD/Core-Shell QD. The ligand usually bears a pendant thiol group for surface attachment although amines and alcohols have also been used. For biolabelling purposes the new ligand will also contain functionality that permits attachment to the intended target and encourages water solubility [5, 10]. Where chemosensing is the aim, the design is even more crucial. The new ligand must not only provide a receptor for the target but it has to be designed in such a way that the binding event causes a perturbation of the QD fluorescence. To date, several systems have been designed that use PET, inner filtering (or competitive absorption) effects and Forster Resonance Energy Transfer (FRET) mechanisms to report the binding event.

QD sensing systems

Sensors based on energy transfer

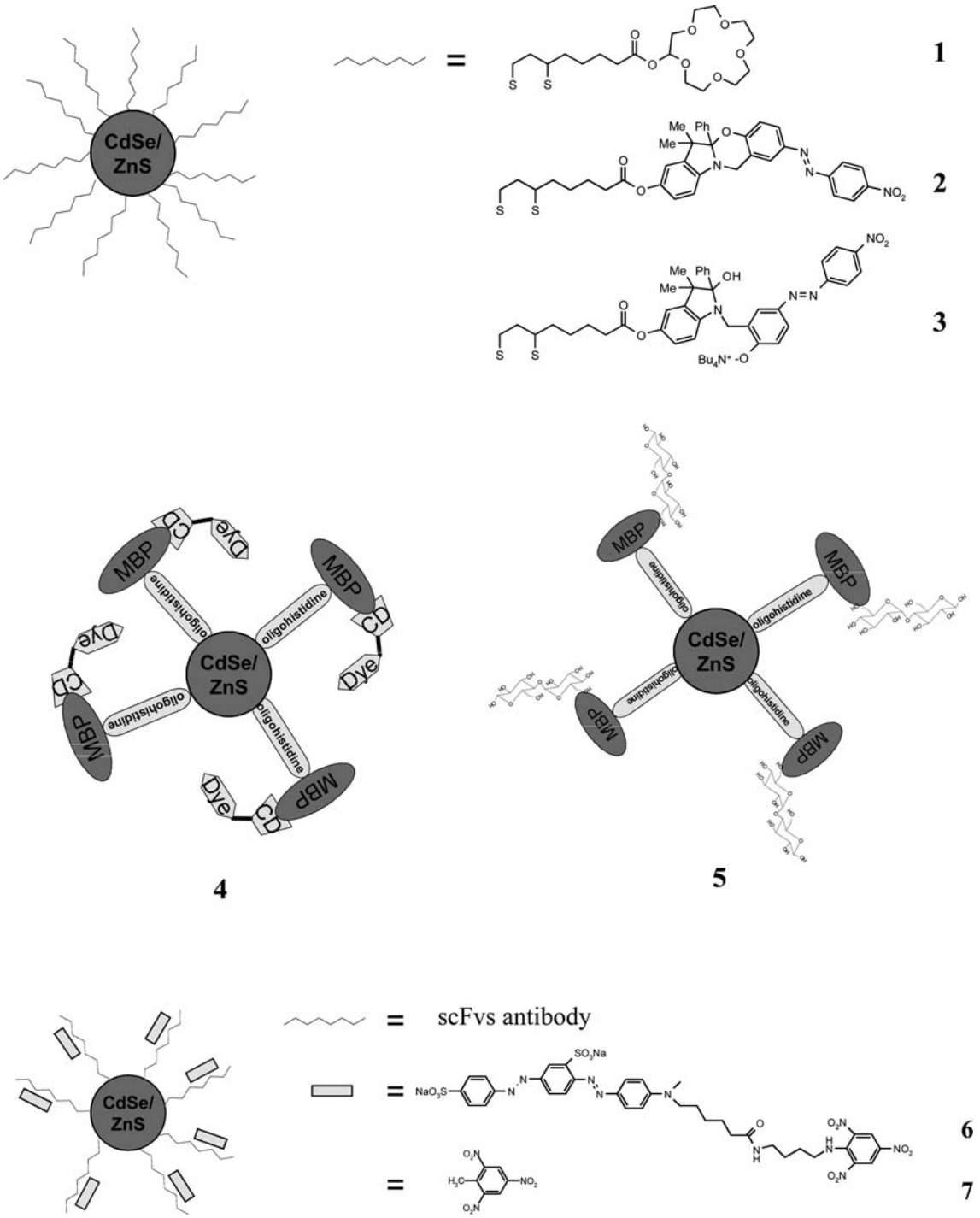
Quantum dots have been utilised effectively as either donors or acceptors in Forster Resonance Energy Transfer (FRET) based luminescent sensors. Their broad absorption spectra make them ideal energy acceptors. Chen *et al*. [11] cleverly devised a CdSe/ZnS dual QD system to measure K^+ ion at the μM level in aqueous solution (**1**). Two different sized quantum dots were employed: a 3.2 nm (em = 545 nm) QD served as the energy donor and a 5.6 nm (em = 635 nm) particle as the energy acceptor. To each was bound a 15-crown-5 receptor using a lipoic acid linker. The crown ether showed selectivity for K^+ and formed a QD (545 nm):K^+:QD (635 nm) sandwich complex upon addition of the metal ion. Formation of the complex resulted in the different sized quantum dots coming close enough together to engage in energy transfer. A ratiometric response was therefore realised upon increasing levels of K^+, the emissions at 545 nm and 635 nm decreasing and increasing respectively. Of course QD (545 nm):K^+:QD (545 nm) and QD (635 nm):K^+:QD (635 nm) complexes are also possible but are statistically less probable (25% each) than the mixed size complex (50%).

The ability of the benzoxazine **2** to convert to the hemiaminal **3** on addition of hydroxide ion was used to develop a CdSe/ZnS QD sensor sensitive to pH [12]. The benzoxazine was again linked to the QD using lipoic acid. In acidic medium **2** shows an absorption band centred at 375 nm which is lost on addition of hydroxide ion and replaced by a band centred at 574 nm by its conversion to **3** (Figure 1). The absorbance maxima of **3** at 574 nm, overlaps well with the emission wavelength of the QD (555 nm). Therefore, the QD acts as an energy donor for **3** and an estimation of solution pH is determined by a reduction in the QD emission. The photoluminescence (PL) of the QD-2 conjugate can be recovered upon addition of trifluoroacetic acid, clearly demonstrating the reversibility of the system. However, the requirement of chloroform as the solvent leads to obvious restrictions.

The addition of Maltose Binding Protein (MBP) to CdSe/ZnS QD's has been used to sense for the disaccharide maltose [13] and the explosive TNT [14]. MBP was bound to a CdSe QD via an oligohistidine tag (**4**), and in the absence of maltose the fluorescence was quenched by addition of an energy accepting non-fluorescent dye bonded to *β*-cyclodextrin. The cyclodextrin sugar binds to the central binding pocket of MBP, and the QD emission quenched by FRET to the dye. Addition of maltose ejects the cyclodextrin–dye complex from the binding pocket by competitive displacement and the fluorescence is restored (**5**). A similar strategy was employed in the detection of TNT. Anti-TNT specific antibody fragments (scFvs) were again attached to the CdSe–ZnS QD's via an oligohistidine sequence. The dye-labelled TNT analogue **6**, binds to the scFv binding/recognition site and quenches QD fluorescence by FRET as before. Addition of TNT (**7**) displaces its dye-labelled analogue, the FRET channel is removed, and fluorescence is recovered.

Sensors based on photoinduced electron transfer (and other mechanisms)

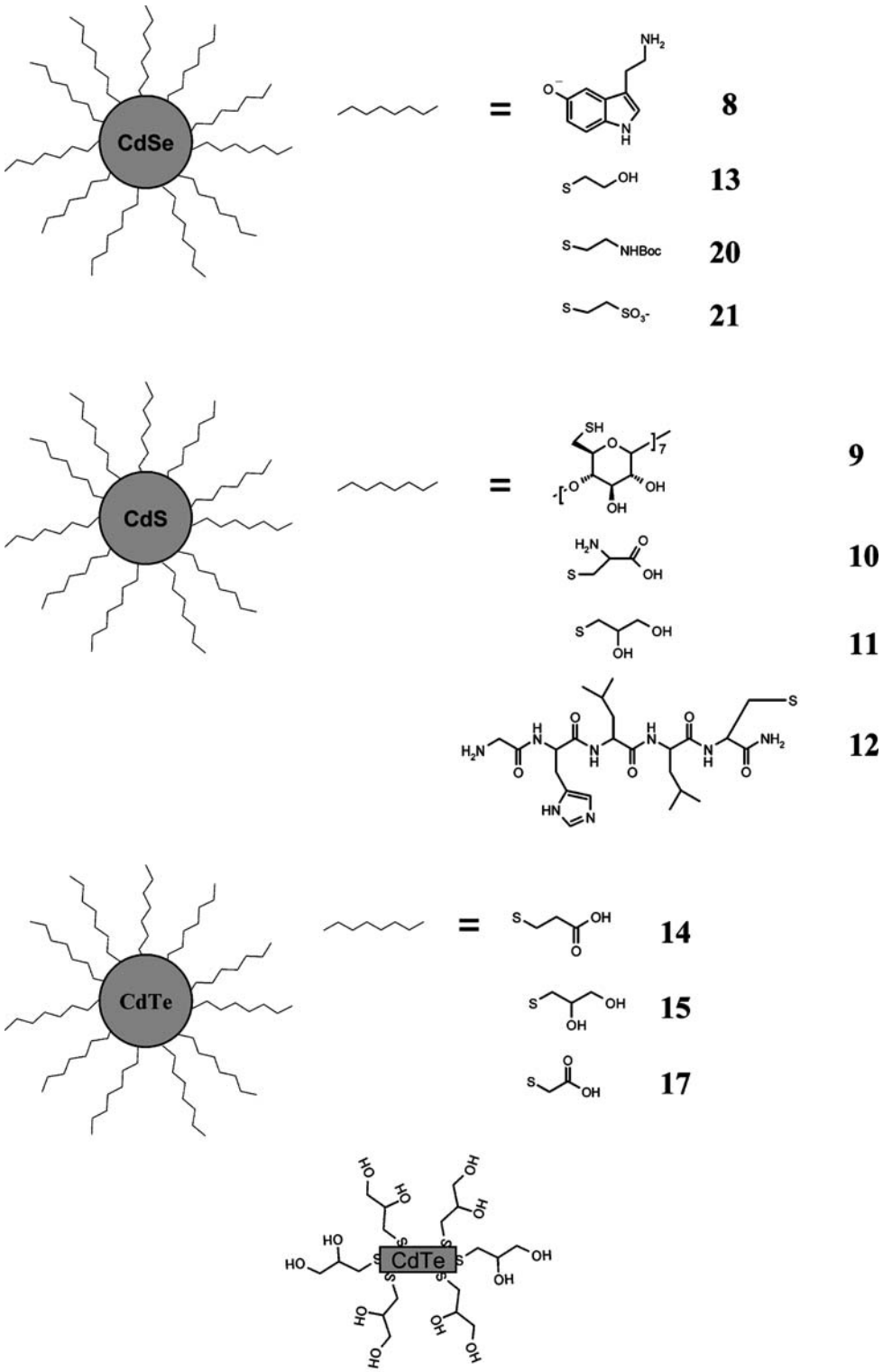
The Photoinduced Electron Transfer (PET) mechanism is central to the design of many luminescent sensors with organic fluorophores [15, 16]. PET has also been used to influence the fluorescence emission of quantum dots. Addition of *p*-phenylenediamine (PPD) to a colloidal solution of CdSe quantum dots resulted in quenching of the fluorescence emission [17]. Upon excitation, PPD (E_{ox} 0.26 eV) reduces a hole in the valence band of the CdSe QD, (band gap 1.2 eV) thus disrupting the radiative recombination process of the electron-hole pair. Addition of *n*-butylamine ($E_{ox} > 1.9$ eV) was shown to enhance the fluorescence emission by passivation of



surface trap sites. However, other studies have shown the same reagent to quench fluorescence by acting as a hole acceptor [18, 19].

A quenching effect was also observed when the neurotransmitter serotonin was bound to CdSe QD's (**8**) via its phenolic group [20]. Distancing the neurotransmitter from the QD shell by the addition of a spacer restored the fluorescent emission.

CdS QD's, with perthiolated β-cyclodextrin ligands (**9**) attached to the surface were used by Palaniappan *et al*. [21] to demonstrate a redox controlled ''On-Off-On'' switching behaviour in aqueous solution. In the absence of analyte, the natural fluorescence of the CdS QD is observed. Addition of the redox active ferrocene quenches the fluorescence most likely by PET to the QD. Fluorescence emission is restored on addition of adamantane carboxylic acid, which, being a better fit for the hydrophobic cyclodextrin cavity ejects the offending ferrocene. The CdS QD used displayed a low luminescence efficiency which was improved upon in a subsequent study by utilising CdSe–CdS core-shell QD's [22]. Calixarenes have also been bound to CdSe–ZnS QD's, however no selectivity for any particular analyte was observed [23].



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The amino acid L-cysteine has been bound to CdS QD's in more than one study (**10**). Chen and Rosenzweig [24], who were first to demonstrate that QD-organic ligand conjugates could be used as quantitative reporters of ion concentration, found that addition of Zn^{2+} ion increased the fluorescence intensity of the CdS-cysteine QD's. Chen and Zhu [25] found the same system to demonstrate selectivity for Ag^{+}, this ion not being tested in the former study. Both systems "switched on" fluorescence on addition of analyte. Chen and Rosenzweig also used thioglycerol as a ligand (**11**) which demonstrated selectivity for Cu^{2+}. Binding Cu^{2+}

resulted in a quenching and a red shift of luminescence emission, attributed to effective electron transfer from the thioglycerol to the Cu^{2+}.

Gattas-Asfura and Leblanc [26] showed that the addition of short peptides to the CdS QD surface resulted in fluorescence being ''switched off'' in the presence of Ag^{+} and Cu^{2+}. The peptide sequence Gly-His-Leu-Leu-Cys showed the best selectivity of those tested (**12**). The Leu-Leu unit was incorporated to provide a hydrophobic sheath to prevent both the metal ions and the basic histidine residue from interfering with the surface of the QD. The Gly-His dipeptide was selected as it previously demonstrated selectivity for Cu^{2+} [27]. Again electron transfer is the likely culprit for the fluorescent quenching, although the visible spectrum of Cu^{2+} means energy transfer is also possible.

The protein Bovine Serum Albumin (BSA) has been bound to CdSe–ZnS QD's and was shown to display selectivity for Cu^{2+} [28]. The CdSe–ZnS BSA conjugate's luminescence was quenched on addition of Cu^{2+} and Fe^{3+} ions in aqueous solution. The quenching of the latter was attributed to an inner filter effect and was cancelled by addition of fluoride ions to form the colourless FeF_6^{3-} complex. The quenching effect of Cu^{2+} was attributed to a surface displacement of Cd^{2+} ions from the surface of the QD by Cu^{2+} ions. The driving force behind this exchange was the increased solubility of CdSe compared to CuSe in the aqueous solvent. CdSe/CdS QD's coated with mercaptoethanol ligands (**13**) [29] and CdTe QD's with mercaptopropionic acid ligands (**14**) [30] also displayed similar quenching effects with Cu^{2+} (Figure 2).

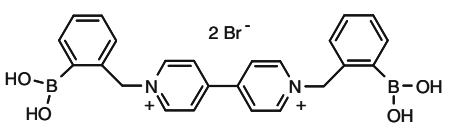
A striking difference in sensitivity was observed between thioglycerol capped CdTe quantum dots and nanorods [31]. Although the same core shell – ligand system was used, the QD's (**15**) demonstrated only a 4% increase in luminescence on addition of Zn^{2+}, compared to a 68% increase for the nanorods (**16**). The selectivity of these particular compounds were poor however, with calcium ion also increasing luminescence, while the addition of manganese, nickel and cobalt ions resulted in luminescence quenching.

CdTe QD's, capped with thioglycolic acid have been prepared by Susha *et al*. [32] and tested for selectivity against a range of metal ions in aqueous solution. No great selectivity was observed but the results were interesting nonetheless. Small amounts (2×10^{-6} to 5×10^{-5} M) of Mg^{2+} and Ca^{2+} led to a 20–40% enhancement of luminescence which was quenched three fold by an increase in the concentration range of between 5×10^{-5} and 5×10^{-4} M. Further increases in concentration had no effect on the luminescence intensity. Both Ag^{+} and Fe^{2+} proved to be very efficient quenchers of the QD fluorescence, the mechanism again most likely being electron transfer from the QD reducing Fe^{2+} to Fe^{+} and Ag^{+} to Ag^{0}. This same study also investigated the effect of pH on the fluorescent emission of the CdTe QD's. No effect on the luminescence intensity was observed on addition of base from pH 12 to pH 6, after which a linear decrease was observed from pH 6 to pH 4, resulting in a 90% quenching of fluorescence.

As mentioned previously, Maltose Binding Protein (MBP) was used in the construction of the FRET sensor **4** for the detection of maltose. The improved maltose sensor prepared by Sandros *et al*. [33, 34] is based on a PET mechanism. A metallothionein protein-MBP conjugate (MT-MBP) was orthogonally bonded to both a CdSe–ZnS QD and a Ru(II) complex. The geometry is such that in the absence of maltose, the Ru(II) complex is close enough to the QD to participate in PET and quench luminescence by reduction of a hole in valence band. On addition of maltose, a lever-action conformational change increases the ''through space'' distance between the Ru(II) complex and the QD, resulting in a cancellation of the PET process and concomitant increase in luminescence intensity is observed. This same study investigated the effect of the ZnS shell on the efficiency of the PET process but found only a slight increase in luminescence intensity on addition of maltose to the CdSe QD's compared to the CdSe–ZnS system. This system has an advantage over **4** as it is a reagentless system, in so far as it does not require the addition of a cyclodextrin–dye complex to achieve luminescence quenching.

Another example of a two component system similar to **4** was employed by Cordes *et al*. for the detection of glucose [35]. Here, a boronic acid substituted viologen (**18**) acts as a quencher/receptor. When added to an aqueous solution of CdSe/ZnS QD's coated with surface amine groups, it quenches luminescence due to PET from the excited QD to the substituted viologen. This type of quenching behaviour has been demonstrated before with methyl viologen [36], but the boronic acid substituted version showed a significant fluorescence re-enhancement upon the addition of D-Glucose, due to the formation of the boronate (**19**) (Figure 3). No selectivity data was provided against other physiological relevant monosaccharides, and the fluorescent enhancement was modest in the physiological range, but again this illustrates that receptors used in traditional organic based sensors can prove effective with QD's also.

Sensing for the toxic and environmentally important cyanide ion has been achieved by Jin *et al*. [37, 38] using



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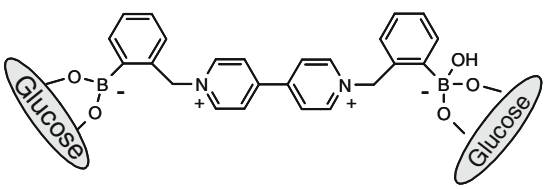

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CdSe QD's. Their initial attempt using N-Boc cysteamine as the ligand (**20**), although successful in quantitatively determining cyanide ion concentration at μM levels, required methanol as the solvent. This was subsequently improved upon using the water soluble 2-mercaptosulfonate ligand (**21**). Both demonstrated a quenching of fluorescence on addition of CN^-.

Conclusion

This review highlighted some of the recent examples of QD-sensor systems based primarily on electron and energy transfer mechanisms. Still in its infancy in terms of sensor research, QD's could some day be as common as, or even supersede organic dyes as the prevalent signalling unit in luminescent sensing systems. The synthesis and chemical adaptability of QD's is becoming more straightforward and offers increasing variability to the chemist. However, it still remains a challenge to produce cheap, robust well functioning QD sensor that are suitable for *in vivo* applications. Nonetheless, if progress continues to be as rapid as it has been in the last 5 years, then the future looks very promising indeed.

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