Selective turn-on fluorescence detection of cyanide in water using hydrophobic CdSe quantum dots†

**Angeles Touceda-Varela, Emily I. Stevenson, José A. Galve-Gasión,
David T. F. Dryden and Juan C. Mareque-Rivas***

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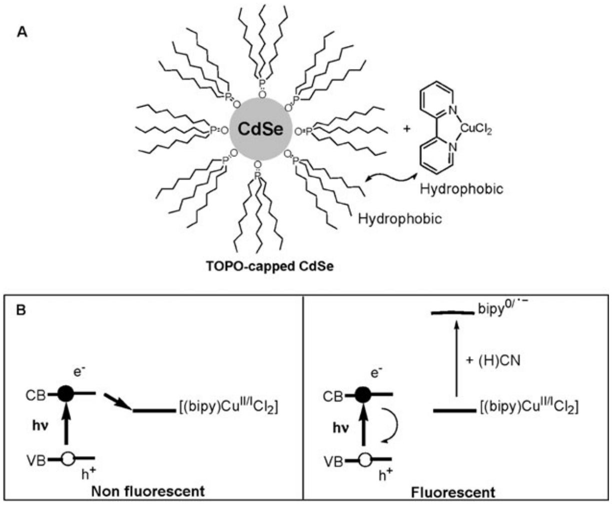
The ability of 2,2′-bipyridine-bound copper(II) ions to quench the photoluminescence of hydrophobic CdSe quantum dots is used to create a novel, selective turn-on fluorescence cyanide sensor.

The development of anion sensors that work in water is a challenging area of considerable current interest.[1,2] Colloidal semiconductor nanocrystals (quantum dots, QDs) have attracted considerable attention in recent years. QDs have been shown to be useful for applications ranging from electronics and photonics to *in vivo* cellular imaging.[3–6] Although some recent studies have shown that the photoluminescence of QDs is sensitive to the presence and type of ions close to the semiconductor core,[7] so far there are very few QD-based anion sensors.[7h,i] One of the most common and readily available QD is CdSe protected with tri-*n*-octylphosphine oxide (TOPO). For ion sensing in water, however, the strategy has been to coat the semiconductor core with ligands capable of inducing ion recognition and water solubility.[7] Often the design and synthesis of these ligands and the derivatization of the QD with them is time consuming, expensive and/or complicated, and in many cases, water-solubilization reduces the photoluminescence efficiency of the nanocrystal. Herein, we report a new approach to create effective fluorescence probes in water based on supramolecular complexes formed by mixing hydrophobic QDs with metal complexes and exploiting photoinduced electron transfer between them.[8] Specifically, we have used the combination of TOPO-coated CdSe QDs, 2,2′-bipyridine(bipy) and $CuCl_2$ (Scheme 1) as a turn-on fluorescence cyanide probe.

Hydrogen cyanide is a chemical warfare agent which affects the ability of cells to utilize oxygen. Although cyanide is extremely toxic (0.5–3.5 mg per kg of body weight are lethal to humans), it finds common industrial use in organic chemicals and polymers such as nitriles, nylon and acrylic plastics, fertilizer factories, precious metal mining and metal plating.[9] As a result, there is considerable interest in finding readily available, simple-to-use and inexpensive methods to detect cyanide.[10] Two recent studies by Sanz-Medel *et al.* have used water-soluble QDs as cyanide probes.[7h,i] Studies carried out on fire survivors and victims have shown blood cyanide levels of 21.6 ± 36.4 μM in the survivors, and 116.4 ± 89.6 μM in the victims.[11]

The TOPO-coated CdSe QDs used in this study were prepared following the literature procedure reported by Peng *et al.*[12] The results reported below are based on CdSe QDs with the first absorption maximum at 574 nm, and a maximum emission peak at 603 nm with excitation at 350 nm, although similar results were obtained with QDs of other sizes (colours). The size of the nanocrystal was estimated to be 3.64 nm from the 574 nm first absorption peak.[13] The QDs were dissolved in chloroform and mixed with 1:1 mixtures of bipy and MCl_2 (M = Fe, Zn, Cu, Co and Ni) in chloroform. The final QD concentration ($\sim$440 nM) was estimated from the size-dependant extinction coefficient of the first absorption peak.[13] Bipy alone did not affect the fluorescence properties of the QDs, however, 1 : 1 mixtures of this readily available chelating ligand and MCl_2 changed the photoluminescence of the CdSe nanocrystals (Fig. 1). The 1 : 1 mixture of bipy and $CuCl_2$, which affords $[(bipy)CuCl_2]$,[14] proved to be the most effective quencher (100% quenching at 20 μM concentration, Fig. 1).

The photoluminescence of QDs arises from the recombination of the electron-hole pair. It has been suggested that the interaction of molecules and ions with the surface atoms of the nanocrystal can affect the efficiency of this recombination, and several mechanisms have been proposed.[7] Among the quenching mechanisms are inner filter effects, non-radiative



Scheme 1 Cyanide QD-based probe (A). Fluorescence quenching and cyanide sensing principle (B).

School of Chemistry, University of Edinburgh, Edinburgh, UK EH9 3JJ. E-mail: juan.mareque@ed.ac.uk; Fax: 0131 6504743; Tel: 0131 6504761

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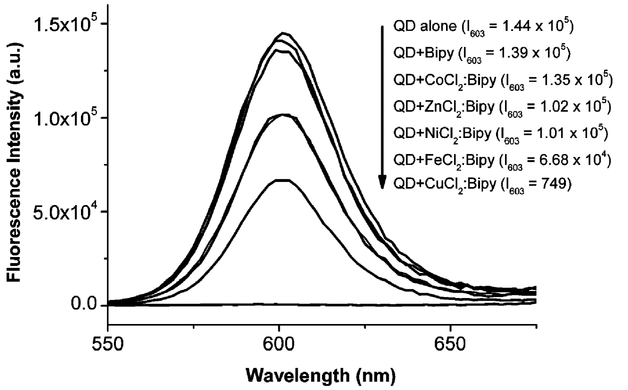

Fig. 1 Effect of 1 : 1 mixtures of MCl_2 and bipy (20 μM) on the photoluminescence of TOPO-coated CdSe QDs (∼440 nM) in chloroform excited at 350 nm.

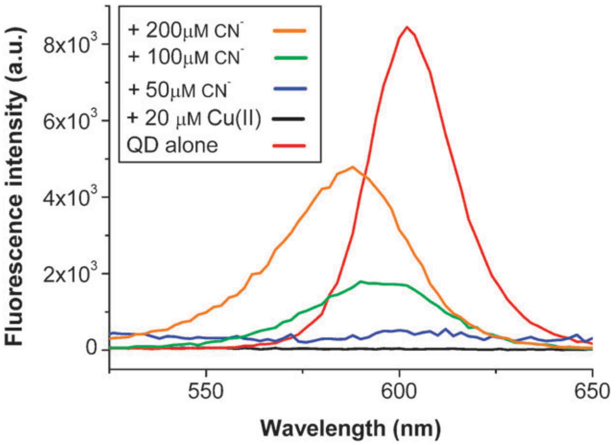

Fig. 2 Photoluminescence of the TOPO-coated CdSe QDs in chloroform (∼440 nM) after mixing with water (50 mM HEPES, pH 7.5) (red line); water solutions (50 mM HEPES, pH 7.5) with 20 μM $[(bipy)CuCl_2]$ (black line); water solutions (50 mM HEPES, pH 7.5) with 20 μM $[(bipy)CuCl_2]$ and 50 μM CN^- (blue line), 100 μM CN^- (green line) and 200 μM CN^- (orange line).

recombination pathways and electron transfer processes, whereas enhancement of pholotuminescence has been attributed primarily to passivation of trap states in the surface. In this case, quenching of the QD photoluminescence is due to electron transfer between the QD and the complex. This is thermodynamically favorable because $[(bipy)CuCl_2]$ has the $Cu^{II/I}$ reduction potential at 0.5 V *vs.* NHE (see Supplementary Information†), which is well above the potential for the excited CdSe QDs (ECB ≈ −1.0 V *vs.* NHE).[15] This was confirmed by NMR studies (see Supplementary Information†). The ^1H NMR spectra of the QD : $[(bipy)CuCl_2]$ mixtures in $CDCl_3$ did not have any signal in the 7–9 ppm region for the bipy protons due to coordination to the paramanetic Cu^{II} ion. Upon irradiation at 350 nm for 2 h, however, the characteristic bipy signals appeared suggesting the conversion to Cu^I. Concerning the approach of the complex to the nanoparticles, it is most likely facilitated by the ability of bipy to form hydrophobic interactions.[16]

Cyanide reacts with copper ions forming very stable $[Cu(CN)_n]^{(n-1)-}$ species.[17] This property has been exploited to remove copper ions from chelating pyridine-based ligands.[18] Recently, we applied this concept to detect cyanide using self-assembled monolayers and electrochemical impedance spectroscopy.[19] Addition of water (50 mM HEPES, pH 7.5) with 100 μM CN^- to the chloroform solution containing the TOPO-coated CdSe QD and 20 μM $[(bipy)CuCl_2]$ restored and blue-shifted (by *ca.* 10 nm) the fluorescence of the QD (Fig. 2).[20] IR studies of the evaporated chloroform solution showed a ν_{CN} bands at 2124 and 2165 cm^{-1} (see Suplementary Information) characteristic of CuCN.[21] Thus, this sensor works by demetallation of the $[(bipy)CuCl_2]$ complex. Bipy, which has a reduction potential of −2.0 V *vs.* NHE (see Suplementary Information†), is not able to quench the QD fluorescence. We investigated the effect of a wide range of other anions at pH 7.5 (50 mM HEPES): NO_3^-, AcO^-, SO_4^{2-}, F^-, Cl^-, Br^-, I^-, ClO_4^-, HCO_3^- and $H_2PO_4^-$. Only cyanide turns on the fluorescence of the CdSe nanocrystals at neutral pH. Thus, the copper-quenched CdSe nanocrystal is a probe with excellent selectivity for cyanide. Unlike any other currently existing cyanide sensor, this simple supramolecular construct provides an extremely simple fluorescent sensor for the detection of the physiological safeguard level of cyanide (20–100 μM) in water, and it works at physiological pH (*i.e.* it can be used to detect $HCN_{(g)}$). In the two water-soluble QD-based cyanide sensors reported to date,[7h,i] cyanide turns off the photoluminescence of the CdSe core. In this case, however, the photoluminescence of the QD is turned on by cyanide, and as a result, the changes are clearly visible by the naked eye even after days (Fig. 3A).[22]

We explored to see if it is possible to avoid the use of toxic, volatile organic solvents like chloroform for $HCN_{(g)}$ detection using this construct. Ideally, we wanted to find a very cheap, accessible and simple method. Polystyrene is a widely used economic polymer soluble in chloroform. Several recent reports have provided elegant methods for the incorporation of QDs onto the surface or inside polymer particles.[23] Polystyrene (PS) was dissolved in a 440 nM chloroform solution of the TOPO-coated CdSe QDs. Evaporation of the solvent produced a strongly fluorescent solid film which was stable for months (Fig. 3B, left). When the QD solution contained a 20 μM 1 : 1 mixture of $CuCl_2$ and bipy, the resulting film was

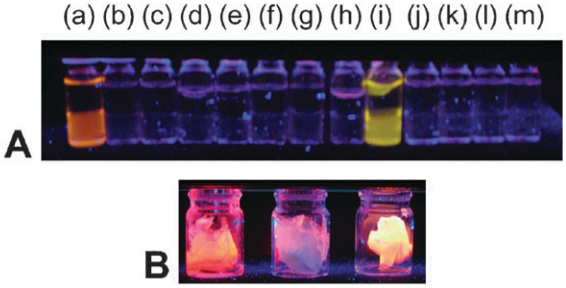

Fig. 3 Naked eye photoluminescence of TOPO-coated CdSe QDs in chloroform under a 365 nm lamp. A; without $CuCl_2$ (a), with 20 μM $CuCl_2$:bipy (b), with 20 μM $CuCl_2$:bipy and 100 μM F^- (c), Cl^- (d), Br^- (e), I^- (f), ClO_4^- (g), NO_3^- (h), CN^- (i), SO_4^{2-} (j), AcO^- (k), $H_2PO_4^-$ (l), HCO_3^- (m). B; PS/QD solid (left), PS/QD/$CuCl_2$:bipy solid after immersion in water (50 mM HEPES pH 7.5) (middle) and 100 μM CN^- (50 mM HEPES pH 7.5) (right).

not fluorescent even after immersion in water (50 mM HEPES, pH 7.5) for several weeks (Fig. 3B, middle). The copper-quenched solid material was immersed into a water solution containing 100 μM CN^- (50 mM HEPES, pH 7.5). Remarkably, after a period of 1–2 days the film showed fluorescence (Fig. 3B, right). The relatively long time required to observe fluorescence may be due to limited access of cyanide to the polymer-immobilized CdSe nanocrystals. However, this result demonstrates that it is possible to form a stable polystyrene QD-based fluorescent probe useful for environmental cyanide monitoring applications. This is another advantage of using water-insoluble QDs. Further studies to develop this proof-of-principle study are currently underway in this laboratory.

In summary, we have combined the ability of $CuCl_2$: bipy mixtures to quench the photoluminescence of TOPO-coated CdSe QDs in chloroform with the ability of CN^- to bind and remove copper ions to create a novel, extremely simple and effective cyanide sensor. Detection of physiologically safe-guard cyanide levels in water is achieved without making the QD water soluble. For the first time a QD probe turns on its fluorescence in the presence of anions. We are exploring several approaches to optimise this and related QD-based sensors for cyanide detection and to exploit the combination of QDs and metal complexes more widely.

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