Cite this: *Chem. Commun.*, 2011, **47**, 7773–7775

www.rsc.org/chemcomm

COMMUNICATION

Detection of toxic mercury ions using a ratiometric CdSe/ZnS nanocrystal sensor†

Leah E. Page, Xi Zhang, Ali M. Jawaid and Preston T. Snee*

Received 12th March 2011, Accepted 27th May 2011

DOI: 10.1039/c1cc11442e

We have developed a strategy for the ratiometric detection of toxic Hg^{2+} ions using a semiconductor nanocrystal energy-transfer donor coupled to a mercury-sensitive “turn-on” dye acceptor. The results demonstrate a new paradigm of toxic metal sensing that resolves the difficulties with the use of semiconductor nanotechnology for this purpose.

The detection of toxic mercury is important as metal ion regulation has a significant effect on health. Dangerous levels of mercury can enter the human body through the consumption of contaminated fish and can cause serious damage to the nervous system.¹ Brain fog, depression, and vision imparities can be caused by the accumulation of mercury in the nervous system. Poisoning by Hg^{2+} has also been known to cause birth defects. As such, developing simple mercury ion sensing schemes is a significant endeavor.

Emissive semiconductor nanocrystals (NCs, or quantum dots) form an ideal base for creating fluorescent detection systems due to their stability and robust luminescent properties compared to organic dyes.² However, well-passivated water-soluble NCs generally have little intrinsic value as sensors as their photophysical properties are not altered by the presence of many analytes (usually organics) in the surrounding medium. The exception to this occurs in the case of metal ions; due to cation exchange,³ even the most robust core/shell NCs are quenched by aqueous elements such as Ag^+ , Pb^{2+} , Fe^{3+} , Cu^{2+} , and Hg^{2+} . Many studies have examined the “turn-off” response of emissive NCs in the context of sensor development;⁴ although, it must be noted that the singular response is non-specific and difficult to calibrate within complex biological environments. For example, how can one distinguish whether NCs are quenched by the presence of toxic metals in a cell when it is known that NCs are notoriously difficult to deliver into cells⁵ to begin with?

Our group and others have reported the development of emissive NC sensors through the manipulation of fluorescent resonant energy transfer (FRET)⁶ from an environmentally insensitive NC donor to a chemically recognizant chromophore.⁷ In our motif, emissive dyes that have chemically sensitive

absorption spectra are conjugated to water-soluble NCs; efficient energy transfer occurs when the absorption of the chromophore overlaps the emission of the NC donor due to environmental variable(s). This manipulation of energy transfer efficiency from an NC donor to an emissive acceptor engenders a ratiometric optical response. Unfortunately, there is a significant issue to address concerning the development of a similar sensing technology for toxic metal ions; as stated previously, some metals are lethal to the emissive properties of even the most well-passivated of aqueous NCs. In this case, our motif is ostensibly useless in a system where the FRET donor is directly and irreversibly quenched by the analyte of interest.

We demonstrate a method of overcoming this deficit with the appropriate choice of a chemically recognizant energy accepting species that is coupled to a CdSe/ZnS NC FRET donor. Specifically, the sensing capability of a thiosemicarbazide-functionalized⁸ rhodamine B dye—CdSe/ZnS NC coupled chromophore has been examined. We have synthesized the mercury sensitive dye *via* a modification of a previously reported procedure;⁸ see ESI for details.† The hydrophobic nature of the dye causes non-specific adsorption to the surfaces of aqueous polymer-encapsulated⁹ CdSe/ZnS FRET donors when the two chromophores are mixed in a co-solvent. The pendant thiosemicarbazide group causes rhodamine B to be optically inert at visible wavelengths due to the disruption of the delocalized electronic structure of the dye as shown schematically in Fig. 1. Upon exposure to mercuric ions, the subsequent desulfurization reaction returns the visible optical properties of the now “turned-on” dye that becomes an efficient energy transfer acceptor to a CdSe/ZnS NC donor. The novelty of this method centers on the fact that the reaction causes the mercuric ions to form highly water-insoluble HgS ; therefore, the system is capable of sensing aqueous Hg^{2+} ions while the optical properties of the NC donor are protected at the same time. As our results demonstrate, a sensing paradigm where a metal analyte can be permanently sequestered and simultaneously trigger an optical response can resolve all of the issues with toxic metal sensing using fluorescent nanocrystals. Our system is also specifically sensitive to mercuric ions as demonstrated in the ESI.†

Our initial studies on mercury sensing focused on chemically conjugating a Hg^{2+} sensitive probe to water soluble CdSe/ZnS NCs. While we were able to synthesize thiosemicarbazide rhodamine dyes with amine functionality for this purpose,

Department of Chemistry, University of Illinois at Chicago, Chicago, IL, USA. E-mail: sneep@uic.edu; Tel: +1 312-413-2566

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c1cc11442e

Fig. 1 Absorption (solid) and emission (dash) spectra of a thiosemicarbazide-functional rhodamine B probe are functions of the presence aqueous of Hg^{2+} ions due to the formation of a delocalized state after mercury de-sulfurizes the thiosemicarbazide group.

such dye derivatives are highly unstable and become absorptive/emissive before they can be used for chemical sensing. Despite this, we found the rhodamine B mercury probe (Fig. 1) dissolved in DMF can simply be mixed with polymer-encapsulated CdSe/ZnS NCs dispersed in water; after stirring overnight, the chromophores are not phase-separated and cannot be isolated with dialysis. We conjecture that the hydrophobic nature of the pendant phenyl group in the thiosemicarbazide functionality allows for non-specific adsorption of the dye to the amphiphilic polymer-coated NCs such that they may be purified without loss of adsorbed dye; similar non-specifically bound NC-dye couples have been studied in the past.¹⁰ After this processing, the dye displays some activation, see Fig. 2. This is attributed to the instability of the dye in solution; we have also found that the coupled chromophores have a shelf-life of only several days after which time the dye becomes activated. At low dye-to-NC molar ratios, the solutions form a homogeneous and non-scattering solution, while at high dye loading levels (>41 dye : NC by mole) the NCs begin to precipitate out of solution.

The thiosemicarbazide functionality was conjugated to a rhodamine B substrate to maximize the overlap of the mercury-activated dye absorption to the ~ 550 nm emission of CdSe/ZnS NCs. Thus, the FRET efficiency from the NC donor to the mercury-activated dye acceptor is a function of the addition of aqueous Hg^{2+} ions; the FRET interaction was confirmed with photoluminescence excitation spectroscopy as shown in the ESI. The FRET efficiency increases with increasing Hg^{2+} concentration due to the larger overlap of the mercury-activated dye absorption with the NC donor emission. Visible changes in emission of our CdSe/ZnS mercury sensor as Hg^{2+} is added to the solution are observed as well as shown in Fig. S7 of the ESI.[†]

Fig. 2 displays the normalized emission spectra of the dye-NC probes as a function of Hg^{2+} exposure. The emission of the NC is decreased while the dye becomes more fluorescent; we attribute this to energy transfer from the NC to the activated dye.

Fig. 2 The normalized emission spectra from an aqueous dye-NC coupled chromophore (dye:NC ratio = 41; blue = 0 M $\rightarrow$ red = 4.0×10^{-5} M) as a function of Hg^{2+} content. A quantity of dye becomes activated with the initial sample processing resulting in dye emission in the control (0 M Hg^{2+}) sample. Inset: The ratiometric response of the sensor.

The ratio of the integrated dye emission divided by the same of the NC was calculated from this data by deconvolving the coupled chromophore spectra into NC and dye components as shown in the inset of Fig. 2. The linear response of the mercury sensor is calibratable over a range of low-levels of mercury exposure although a roll-off from linearity is observed when the added mercury ion concentration approaches the dye loading levels. This may be attributed to the loss of reactive dye or perhaps by direct quenching of the NC FRET donors by mercury as discussed below. Furthermore, the slope of the calibration ($1.6 \pm 0.4 \times 10^4 \text{ M}^{-1}$) is proportional to the molar dye : NC ratio as shown in the supporting information; reducing the dye : NC ratio by ~ 4 results in a change in the responsivity by nearly the same factor.

A major goal of this study is to demonstrate a sensing motif that resolves the issue of the direct quenching of the donor NC by the analyte of interest, which is self-defeating to a FRET-based ratiometric detection mechanism. In our system, the thiosemicarbazide-functional dye is a “turn-on” chromophore due to its reaction with mercuric ions to form HgS , a highly water-insoluble mineral. We have determined that HgS cannot quench NCs; as such, the dye plays a dual role as the component of a ratiometrically reporting chromophore and as a mercury-sequestering protector of the nanocrystal. Shown in Fig. 3 are the relative quantum yields of a blank NC sample (green) and a coupled dye-NC chromophore (blue dash, dye : NC ratio = 41) as a function of the addition of mercury, normalized to the starting emission intensity. Initially, the dye-NC sensor has a sharper decrease in total fluorescence due to mercury exposure than that observed with the neat NC sample; although, at higher loading levels, the coupled chromophore is more robust than the unfunctionalized NCs. While somewhat perplexing, we realized that the fact that the acceptor rhodamine has a finite quantum yield ($\sim 10\%$) represents an energy sink that initially results in more excitation loss than that caused by mercury-induced NC-donor quenching. To account for this effect,

Fig. 3 The quantum yield QY of blank NCs and coupled dye-NC chromophores as a function of added Hg^{2+} divided by the initial quantum yield $\text{QY}(0)$. The green line is the response of uncoupled ("blank") NCs; also shown are the normalized integrated emissions of dye-coupled NCs with correction for the finite quantum yield of the organic rhodamine component (red) and without (blue-dash).

the sensor emission profiles were separated into NC and dye components. The dye-only spectra were augmented in proportion to the dye's quantum yield, which was then added back to the NC component to simulate a 100% acceptor-efficient system. These total integrated emission spectra are shown in Fig. 3 as the "corrected" relative quantum yields (red line). These data demonstrate that the coupled chromophore is more robust than the neat NCs when exposed to donor-quenching mercuric ions at all concentration levels; furthermore, the level of "protection" scales with the dye:NC ratio as demonstrated in the supporting information. Unfortunately, the fact that the corrected relative quantum yields still drop with mercury exposure shows that our protection scheme is not perfect. Energy is still being lost due to direct quenching of the donor NC by mercury ions even at the highest dye:NC loading ratio; however, we believe that the results demonstrate the success of a new paradigm of toxic metal sensing with semiconductor NCs. Higher dye:NC coupling ratios with brighter and more reactive metal-sensing chromophores will resolve these issues; fortunately, the development of metal-sensing dyes is an active area.¹¹

The calculated analyte detection limit of the dye-NC chromophore (79 ± 2 ppb) is reduced compared to the neat dye alone (1.1 ± 0.2 ppb). This is attributed to the fact that the dye-NC sensor response has a finite background emission, imperfect FRET efficiency, and direct quenching of the NC FRET donor. Despite these limitations, ratiometric sensing with coupled dye-NC chromophores has several advantages over the use of the neat dye alone such as their "self-calibratable" sensing in complex environments,^{7e} ease of photoexcitation of the NC-donor, and the fact that large dye:NC ratios can reduce potential issues with photobleaching. Furthermore, the detection limits of ratiometric sensing schemes may be improved by diluting the sensor to reduce background.

In summary, we have developed a coupled dye-nanocrystal ratiometric FRET sensor that is capable of detecting aqueous Hg^{2+} ,

a toxic metal that directly quenches semiconductor NCs. The specific choice of a mercury-reactive dye component that sequesters the metal in the form of highly water-insoluble HgS mitigates the quenching of the NC-FRET donor. The method of preparation is significantly easier compared to procedures to create all-organic ratiometrically reporting dyes and produces materials that have all the photochemical robustness of inorganic semiconductor NCs. These results also demonstrate that the coupling of a "turn-on" organic component that simultaneously protects the NC fluorophore is a functional paradigm for creating self-calibrating sensors for toxic metals. There are several metal-reactive organic motifs available for developing similar sensing schemes, such as rhodanine and its derivatives¹² that have been used to selectively precipitate NC-quenching ions such as copper from solution and may be easily functionalized for conjugation to NC surfaces. As a result, the sensor motif presented here can be extended towards the robust detection of a variety of toxic ions using semiconductor nanotechnological constructs. This work was funded by the University of Illinois at Chicago.

Notes and references

- 1 T. W. Clarkson and L. Magos, *Crit. Rev. Toxicol.*, 2006, **36**, 609–662.
- 2 R. C. Somers, M. G. Bawendi and D. G. Nocera, *Chem. Soc. Rev.*, 2007, **36**, 579–591.
- 3 D. H. Son, S. M. Hughes, Y. D. Yin and A. P. Alivisatos, *Science*, 2004, **306**, 1009–1012.
- 4 (a) H. Y. Xie, H. G. Liang, Z. L. Zhang, Y. Liu, Z. K. He and D. W. Pang, *Spectrochim. Acta, Part A*, 2004, **60**, 2527–2530; (b) M. T. Fernandez-Arguelles, W. J. Jin, J. M. Costa-Fernandez, R. Pereiro and A. Sanz-Medel, *Anal. Chim. Acta*, 2005, **549**, 20–25; (c) Y. S. Xia, C. Cao and C. Q. Zhu, *J. Lumin.*, 2008, **128**, 166–172; (d) Y. S. Xia and C. Q. Zhu, *Talanta*, 2008, **75**, 215–221; (e) H. Goncalves, C. Mendonca and J. da Silva, *J. Fluoresc.*, 2009, **19**, 141–149.
- 5 (a) I. L. Medintz, T. Pons, J. B. Delehanty, K. Susumu, D. W. Pang, P. E. Dawson and H. Mattoussi, *Bioconjugate Chem.*, 2008, **19**, 1785–1795; (b) J. B. Delehanty, H. Mattoussi and I. L. Medintz, *Anal. Bioanal. Chem.*, 2009, **393**, 1091–1105.
- 6 T. Förster, *Ann. Phys.*, 1948, **437**, 55–75.
- 7 (a) E. R. Goldman, I. L. Medintz, J. L. Whitley, A. Hayhurst, A. R. Clapp, H. T. Uyeda, J. R. Deschamps, M. E. Lassman and H. Mattoussi, *J. Am. Chem. Soc.*, 2005, **127**, 6744–6751; (b) D. M. Willard, T. Mutschler, M. Yu, J. Jung and A. Van Orden, *Anal. Bioanal. Chem.*, 2006, **384**, 564–571; (c) P. T. Snee, R. C. Somers, G. Nair, J. P. Zimmer, M. G. Bawendi and D. G. Nocera, *J. Am. Chem. Soc.*, 2006, **128**, 13320–13321.
- 8 (a) Y. K. Yang, K. J. Yook and J. Tae, *J. Am. Chem. Soc.*, 2005, **127**, 16760–16761; (b) Y. K. Yang, S. K. Ko, I. Shin and J. Tae, *Nat. Protoc.*, 2007, **2**, 1740–1745.
- 9 (a) X. Y. Wu, H. J. Liu, J. Q. Liu, K. N. Haley, J. A. Treadway, J. P. Larson, N. F. Ge, F. Peale and M. P. Bruchez, *Nat. Biotechnol.*, 2003, **21**, 41–46; (b) T. Pellegrino, L. Manna, S. Kudera, T. Liedl, D. Koktysh, A. L. Rogach, S. Keller, J. Radler, G. Natile and W. J. Parak, *Nano Lett.*, 2004, **4**, 703–707.
- 10 (a) A. Boulesbaa, Z. Q. Huang, D. Wu and T. Q. Lian, *J. Phys. Chem. C*, 2010, **114**, 962–969; (b) A. Boulesbaa, A. Issac, D. Stockwell, Z. Huang, J. Huang, J. Guo and T. Lian, *J. Am. Chem. Soc.*, 2007, **129**, 15132–15133.
- 11 (a) E. M. Nolan, S. C. Burdette, J. H. Harvey, S. A. Hilderbrand and S. J. Lippard, *Inorg. Chem.*, 2004, **43**, 2624–2635; (b) S. C. Burdette and S. J. Lippard, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, **100**, 3605–3610; (c) Q. W. He, E. W. Miller, A. P. Wong and C. J. Chang, *J. Am. Chem. Soc.*, 2006, **128**, 9316–9317.
- 12 T. J. Muller and U. Bunz, *Functional Organic Materials: Syntheses, Strategies and Applications*, Wiley-VCH, Weinheim, 2007.