J|A|C|S
COMMUNICATIONS

A Ratiometric CdSe/ZnS Nanocrystal pH Sensor

Preston T. Snee,[†] Rebecca C. Somers, Gautham Nair, John P. Zimmer, Moungi G. Bawendi,* and
Daniel G. Nocera*
Department of Chemistry, Massachusetts Institute of Technology, 77 Massachusetts Avenue,
Cambridge, Massachusetts 02139-4306

Received March 20, 2006; E-mail: mgb@mit.edu; nocera@mit.edu

Semiconductor nanocrystals (NCs) serve as useful fluorescent labels owing to their photostability, continuous absorption spectra, and efficient, narrow, and tunable emission.[1−3] These properties of NCs have been exploited for applications in biological imaging[4] and in single particle tracking studies.[5] Whereas NCs are useful in identifying position in a microenvironment, their intrinsic insensitivity to the presence of most biological or chemical agents renders them of limited utility as sensing probes of that microenvironment. An important step in this direction has been the recent development of water-soluble CdSe NCs with the ability to sense target analytes irreversibly using fluorescence resonant energy transfer (FRET) as a signal transduction mechanism.[6−8] These previous studies, however, do not demonstrate reversible and ratiometric chemical sensing using fluorescent NCs. We now show that a reversible NC-based fluorescent sensor can be designed by conjugating a dye with an equilibrium response to an analyte to the surface of a CdSe/ZnS NC. We show that properly controlling energy transfer between the NC and dye engenders a general method for the development of ratiometric NC sensors, thus providing a means for detecting analytes with high precision, irrespective of changes in excitation intensity, wavelength, or collection efficiency. Our constructs thus remove several difficulties encountered with dye-only-based ratiometric nanoscopic sensing systems.[9]

High quality CdSe NCs overcoated with ZnS and capped with trioctylphosphine oxide ligands[10,11] were encapsulated by a hydrophobically modified poly(acrylic acid) (Figure 1).[12] The squaraine dye[13] shown in Figure 1 was linked to the polymer backbone via ester linkages using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as the coupling agent. We have also found that the same dye conjugation method may be applied to water-soluble NCs that have been cap exchanged with thiol-functionalized poly(ethylene glycol)s.[14] The samples can be easily purified with dialysis filters to ensure that unreacted dyes are removed from the sample. The complete procedures for the synthesis of CdSe/ZnS NCs and squaraine dye and their subsequent conjugation are given in the Supporting Information (SI).

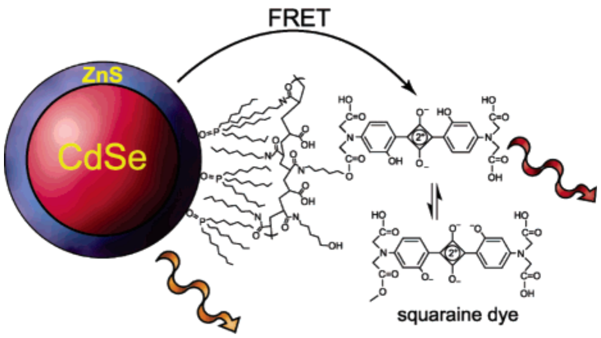

Figure 1. A sensor constructed from a colloidal CdSe NC that is overcoated with an outer layer of ZnS. The native phosphine oxide ligands are encapsulated with an amphiphilic polymer upon which a pH-sensitive squaraine dye is conjugated. Upon excitation, the CdSe/ZnS nanocrystal may either fluoresce or transfer energy to the squaraine dye. The FRET efficiency is modulated by the environment as the dye's absorption profile is a function of pH. Consequently, the ratio of NC to dye emission becomes a function of environmental variables.

The sensing action of the NC−squaraine conjugate is imparted by modulation of the FRET efficiency arising from the engineered overlap of the pH-sensitive dye absorption spectrum with the (pH-insensitive) quantum dot emission (Figure S1 of SI). Owing to the pH dependence of the dye absorption spectrum, the spectral overlap between the dye absorption and the NC emission increases as the pH is lowered. This spectral overlap integral J is directly related to the critical FRET distance, R_o, which in turn affects the energy transfer efficiency $E(R_o)$ as shown in eq 1:

$$J(\mathrm{pH}) = \int \mathrm{d}\lambda \epsilon_{\mathrm{pH}}(\lambda)\mathrm{d}(\lambda)\lambda^4,\ R_o \propto J^{1/6},\ E(R_o) = \frac{R_o^{\ 6}}{R_o^{\ 6} + R^6} \quad (1)$$

where λ is wavelength, $\epsilon_{\mathrm{pH}}(\lambda)$ is the pH-dependent molar absorptivity, $\mathrm{d}(\lambda)$ is the normalized NC donor emission, and R is the donor−acceptor distance. The inset of Figure S1 shows a calculation of R_o as a function of pH; details of the FRET calculation are included in the SI. At high pH, FRET should be inefficient as the spectral overlap is small. Hence, the emission spectrum should be dominated by luminescence from the NC. As the pH is lowered, R_o grows larger and the FRET efficiency increases owing to a strengthening of the dye absorption cross section $\epsilon_{\mathrm{pH}}(\lambda)$. The NC emission should then be quenched as a result of energy transfer to the dye, which will in turn become more emissive.

The experimental results shown in Figure 2 are consistent with the calculated trend in R_o and expected FRET efficiency. The inset shows the response of the absorption profile of the NC−dye conjugate above and below the pK_a (~8.5) of the dye. As observed for the free dye in homogeneous solution (Figure S1), the dye absorption band is suppressed under basic conditions. Consequently, energy transfer from the NC to the dye is inefficient, and the emission spectrum is dominated by the NC at 613 nm. As pH is lowered, the absorption cross section of the dye is increased, and FRET from the NC to the dye becomes more efficient; emission from the NC−dye conjugate is now dominated by that of the dye at 650 nm. The largest changes occur near the pK_a of the dye. The steady-state emission results are confirmed by time-resolved emission studies of the NC−dye conjugate. As shown in Figure S3, NC emission of a conjugated sensor has a reduced lifetime as compared to that of the unconjugated NC. These results establish that the emission from the NC donor is quenched by the presence of the organic dye acceptor, as has been previously observed in other constructs.[6,7]

[†] Present address: Department of Chemistry, The University of Illinois at Chicago, Chicago, IL 60607.

10.1021/ja0618999 CCC: $33.50

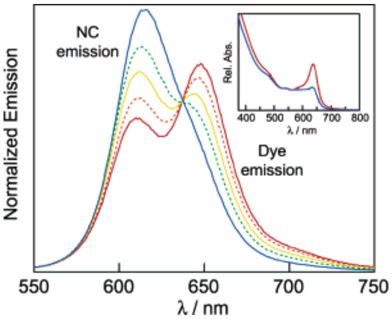

Figure 2. The emission profile of a water-soluble (3.2 nm radius) NC−squaraine dye conjugate changes as a function of pH (red solid line, 6.0; orange dotted line, 7.0; yellow solid line, 8.0; green dotted line, 9.0; and blue solid line, 10) with $\lambda_{ex} = 380$ nm. The normalized spectra show pH dependence with an isosbestic point appearing at 640 nm. The absorbance of the squaraine dye is suppressed in the conjugate at basic pHs as shown in the inset. The overall quantum yield of this construct is 7 ± 1%. Deconvolution of the absorption spectra into dye and NC components reveals that the dye to NC ratio is (3.4 ± 0.2):1. Shown in Figure S2 of the SI are the data from a 7.5:1 dye to NC conjugated sensor.

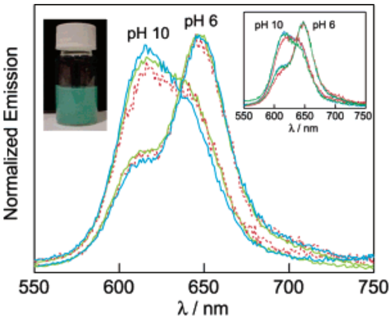

Figure 3. Sensing of the local pH by the NC/dye construct with variations in the excitation intensity and local environment. The ratio of NC to dye emission varies such that pH is determined within 5% when altering the slit entrance of the Xe lamp excitation of the fluorimeter (red dotted line, 0.5 mm; green solid line, 2.00 mm) and when examining the construct within a highly scattering media (blue solid line) shown by the picture of the vial. The inset shows that emission was independent of excitation wavelength (blue solid line, 380 nm; green solid line, 450 nm; red solid line, 520 nm).

The modulation of the FRET efficiency by pH results in an emission dependence between the NC and dye that is naturally ratiometric. Notably, the change in the NC−dye emission occurs about an isosbestic point at 640 nm. The solution pH can be read out precisely by taking the ratio of each emission peak intensity (NC and dye) to the intensity at the isosbestic point, which functions as an internal reference (or by measuring the ratio of any two unique points). This ratiometric approach is powerful when compared to typical chemo- and biosensors that display a single intensity-based response to analytes (i.e., either brightening or darkening) because the ratiometric construct is not sensitive to fluctuations of light excitation or collection efficiency as sensing is self-referencing.[15,16]

To highlight the importance of the self-referencing capability of our constructs compared to other organic sensing nanomaterials, we have examined the results of modulating the excitation intensity and wavelength on the normalized fluorescent spectra of the sensor in pH 6 and pH 10 buffered solutions. In separate experiments, ∼130 nm silica microspheres were added to the solutions to produce a highly scattering medium. The results are shown in Figure 3. Using the ratio of NC to dye emission in clear solutions as a calibration, we are able to determine the pH within 5% when altering the excitation intensity over ∼1 decade and varying the composition of the environment. Such precision is impossible to achieve for the dye alone. In addition, the continuous absorption manifold of the CdSe NCs results in a excitation-wavelength-independent band shape (see inset, Figure 3), a feature that is not present in single molecular ratiometric sensors.[17] Thus, the NC−dye conjugate does not need to use two independent excitation sources (or alternatively a single excitation specifically at an absorptive isosbestic point) for proper function.

In summary, we have developed a new strategy for chemical and biological sensing by tethering emissive water solubilized NCs to environmentally sensitive dye molecules. We have observed a ratiometric response to pH owing to modulation of FRET efficiency between the emissive NC and dyes conjugated to the NC surface. The approach is general as a sensing construct because the narrow, size-tunable emission spectrum of NCs enables them to be FRET donors that may be easily custom-engineered to match the acceptor absorption features of a dye conjugated to the surface of the NC. Taken together with the broad excitation spectrum and photostability conferred by NCs, the reversible and ratiometric approach presented here makes NCs versatile agents for chemical and biological sensing.

Acknowledgment. The authors would like to thank Y. Chan for helpful discussions. This work made use of the MRSEC Shared Facilities supported by the NSF under Award Number DMR-0213282, as well as the Harrison Spectroscopy Laboratory (NSF-011370-CHE). M.G.B. and D.G.N. acknowledge support from a Collaborative Research in Chemistry Grant by the NSF (NSF-CHE-020989). M.G.B. also acknowledges support from the NIH funded MIT−Harvard NanoMedical Consortium (1U54-CA119349, a Center of Cancer Nanotechnology Excellence), and the ARO through the Institute for Collaborative Biotechnologies. D.G.N. acknowledges support from Corning Inc.

Supporting Information Available: Full synthetic procedures and NMR spectra for the preparation of pH-sensitive squaraine dyes, water solubilized core shell NCs, and the conjugation of the two. Time-resolved emission of NC versus NC/squaraine dye conjugates as well as results from other pH-sensing conjugates. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dahan, M.; Levi, S.; Luccardini, C.; Rostaing, P.; Riveau, B.; Triller, A. *Science* **2003**, *302*, 442.
(2) Michalet, X.; Pinaud, F. F.; Bentolila, L. A.; Tsay, J. M.; Doose, S.; Li, J. J.; Sundaresan, G.; Wu, A. M.; Gambhir, S. S.; Weiss, S. *Science* **2005**, *307*, 538.
(3) Lidke, D. S.; Nagy, P.; Heintzmann, R. Arndt-Jovin, D. J.; Post, J. N.; Grecco, H. E.; Jares-Erijman, E. A.; Jovin, T. M. *Nat. Biotechnol.* **2004**, *22*, 198.
(4) Alivisatos, A. P. *Nat. Biotechnol.* **2004**, *22*, 47.
(5) Pouya, S.; Koochesfahani, M.; Snee, P. T.; Bawendi, M. G.; Nocera, D. G. *Exp. Fluids* **2005**, *39*, 784.
(6) Goldman, E. R.; Medintz, I. L.; Whitley, J. L.; Hayhurst, A.; Clapp, A. R.; Uyeda, H. T.; Deschamps, J. R.; Lassman, M. E.; Mattoussi, H. *J. Am. Chem. Soc.* **2005**, *127*, 6744.
(7) Clapp, A. R.; Medintz, I. L.; Mauro, J. M.; Fisher, B. R.; Bawendi, M. G.; Mattoussi, H. *J. Am. Chem. Soc.* **2004**, *126*, 301.
(8) Medintz, L.; Konnert, J. H.; Clapp, A. R.; Stanish, I.; Twigg, M. E.; Mattoussi, H.; Mauro, J. M.; Deschamps, J. R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9612.
(9) Sumner, J. P.; Aylott, J. W.; Monson, E.; Kopelman, R. *Analyst* **2002**, *127*, 11.
(10) Murray, C.; Norris, D. J.; Bawendi, M. G. *J. Am. Chem. Soc.* **1993**, *115*, 8706.
(11) Dabbousi, B. O.; Rodriguez-Viejo, J.; Mikulec, F. V.; Heine, J. R.; Mattoussi, H.; Ober, R.; Jensen, K. F.; Bawendi, M. G. *J. Phys. Chem. B* **1997**, *101*, 9463.
(12) Wu, X.; Liu, H.; Liu, J.; Haley, K. N.; Treadway, J. A.; Larson, J. P.; Ge, N.; Peale, F.; Bruchez, M. P. *Nat. Biotechnol.* **2003**, *21*, 41.
(13) Isgor, Y. G.; Akkaya, E. U. *Tetrahedron Lett.* **1997**, *38*, 7417.
(14) Uyeda, H. T.; Medintz, I. L.; Jaiswal, J. K.; Simon, S. M.; Mattoussi, H. *J. Am. Chem. Soc.* **2005**, *127*, 3870.
(15) Liu, J.; Diwu, Z.; Leung, W. Y. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2903.
(16) Rink, T. J.; Tsien, R. Y.; Pozzan, T. *J. Cell. Biol.* **1982**, *95*, 189.
(17) Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440.

JA0618999