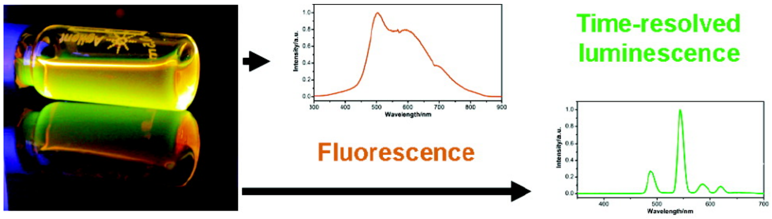
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

Incorporating Lanthanide Cations with Cadmium Selenide Nanocrystals: A Strategy to Sensitize and Protect Tb(III)

Demetra A. Chengelis, Adrienne M. Yingling, Paul D. Badger, Chad M. Shade, and Stphane Petoud

J. Am. Chem. Soc., **2005**, 127 (48), 16752-16753• DOI: 10.1021/ja0511725 • Publication Date (Web): 11 November 2005



Journal of the American Chemical Society is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

J|A|C|S
COMMUNICATIONS

Published on Web 11/11/2005

Incorporating Lanthanide Cations with Cadmium Selenide Nanocrystals: A Strategy to Sensitize and Protect Tb(III)

Demetra A. Chengelis, Adrienne M. Yingling, Paul D. Badger, Chad M. Shade, and Stéphane Petoud*

Department of Chemistry, The University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, Pennsylvania 15260

Received February 24, 2005; E-mail: spetoud@pitt.edu

As biological assays based on luminescence become more prevalent, the demand increases for reporters with properties such as strong resistance to photobleaching and a signal which can be discriminated from background fluorescence (autofluorescence). The narrow, well-defined emission bands and long luminescence lifetimes of lanthanide cations make them more desirable than organic fluorophores as reporters.[1−4] To exploit the luminescent properties of lanthanide cations, they need to be sensitized by a suitable "antenna".[5,6] Sensitization has typically been accomplished by coordinating organic ligands having high molar absorptivities to luminescent lanthanide cations. One of the inherent limitations of this approach is that organic ligands possess high frequency vibrations that often deactivate the excited states of the lanthanide cations, or provide poor protection from solvent vibrations, decreasing luminescence intensity.[7,8] We tested a novel strategy to remove these limitations by using luminescent semiconductor CdSe nanocrystals as an antenna to sensitize and protect Tb^{3+}. This work demonstrates that combining Tb^{3+} with CdSe semiconductor nanocrystals (**SCNC**s) creates an advantageous new type of lanthanide-based luminescent material.

In recent developments, nanoparticles have shown that they can play an important role as biological reporters.[9−14] **SCNC**s have unique photophysical properties arising from their quantum confinement effects. For example, the emission band maximum is dependent on nanocrystal size and displays a bathochromic shift as the nanocrystal radius increases.[15] **SCNC**s also display a broad continuum of absorbance wavelengths with large epsilon values, which provides a wide excitation range.[14] In addition, they are highly photostable,[12] can be used in highly dilute solutions, and have a long shelf life. **SCNC**s have been used in a variety of biological applications, such as imagery, replacing traditional organic fluorophores.[13,14] Although the emission bands of CdSe **SCNC**s are more narrow than typical organic fluorophores, they are still broader than those of lanthanide cations.

Lanthanide cations have three main advantages for biological applications: long luminescence lifetimes (micro- to millisecond), which allows autofluorescence removal through time-resolved measurements,[4] sharp emission bands,[1] and insensitivity to photobleaching. Since transitions within the f-orbitals are Laporte forbidden, free lanthanide cations have extremely low extinction coefficients. To overcome this limitation, a sensitizer (antenna) is attached to the lanthanide cation, which harvests light and transfers energy to excite the metal ion.

A new class of reporters with synergistic properties is synthesized by using the **SCNC** structure to sensitize and protect lanthanide cations. The **SCNC**s' energy levels can be tuned, enabling efficient matching to the various accepting levels of different lanthanide cations. As antennae, nanocrystals can minimize luminescence quenching by isolating the lanthanide cation from high frequency vibrations. Unlike organic molecules,[7,8] **SCNC**s possess only low energy lattice vibrations that are less efficient in deactivating the lanthanide cations' excited states. The CdSe:Tb **SCNC**s were synthesized following procedures inspired by Peng et al.[16,17] and Strouse et al.[18] The detailed procedure is described in the Supporting Information. Growth times of the nanocrystals have been chosen in order to maximize the luminescence intensity of Tb^{3+}; 30 s was found to be optimal for the experimental conditions. The synthesized CdSe:Tb nanocrystals were visualized with transmission electron microscopy (TEM); see Figure 1a. Purified and isolated samples dissolved in hexane were aerated onto carbon-coated copper grids for imaging. The sample used for the TEM image was also analyzed by energy dispersive X-ray spectroscopy (EDS). The data reported in Figure 1b indicate the nanocrystals are comprised of Cd, Se, and the dopant[18] Tb.

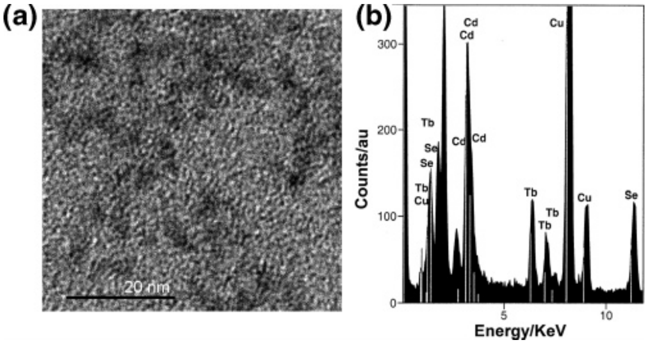


Figure 1. (a) TEM image of the CdSe:Tb nanocrystals, taken at a magnification of 850 K. The size bar represents 20 nm. (b) Energy dispersive X-ray spectroscopy (EDS) analysis of these nanocrystals. The Cu peaks are generated from the copper grid support used for imaging.

The CdSe:Tb **SCNC**s of different growth times display a bathochromic shift (Figure S1, Supporting Information), which demonstrates that doping does not interfere with the quantum confinement properties. Steady-state and time-resolved emission and excitation spectra were collected for the CdSe:Tb nanocrystal aliquots. The emission arising from the lanthanide cation can be discriminated from the nanocrystal luminescence through time-resolved measurements using a typical delay after flash of 200 μs. The total emission of the nanocrystals as well as the Tb^{3+} specific emission was quantified and analyzed for different samples, leading to several interesting findings. In steady-state mode, the total emission of the CdSe:Tb **SCNC**s appears as a relatively broad band (orange, Figure 2). This can be explained by the short material growth time of samples used for this photophysical investigation, leading to a polydisperse size distribution of nanocrystals. Longer growth times and alternative synthetic methods should lead to less dispersity and thus sharper emission bands. Nevertheless, the Tb^{3+} emission appears as narrow, atom-like luminescence. The corresponding excitation spectrum (black, Figure 2) displays a sharp maximum around 300 nm and appears as an asymmetrical band.

10.1021/ja0511725 CCC: $30.25 © 2005 American Chemical Society

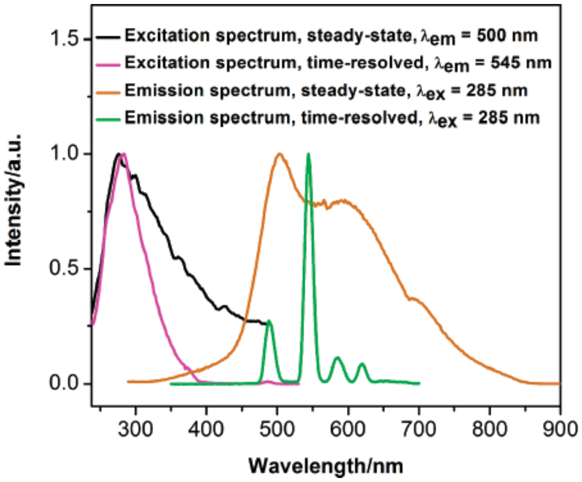


Figure 2. CdSe:Tb **SCNCs** (in chloroform): normalized steady-state and time-resolved excitation and emission spectra. The Tb^{3+} emission is in the same range as the nanocrystal emission; however, it is easily distinguished through time-resolved measurements.

The time-resolved emission spectrum revealed the four main observable Tb^{3+} transitions ($^5D_4 \rightarrow {}^7F_J$, $J = 6, 5, 4, 3$ by decreasing order of energy, green in Figure 2). The time-resolved excitation spectrum (magenta, Figure 2) monitoring the Tb^{3+} $^5D_4 \rightarrow {}^7F_5$ transition ($\lambda_{em} = 545$ nm) revealed a profile that resembles the steady-state excitation spectrum and differs from the excitation profile of the lanthanide salt $Tb(NO_3)_3$ (see Figure S2, Supporting Information). These findings confirm that the nanocrystal structure is acting as an antenna for Tb^{3+}; that is, Tb^{3+} emission is sensitized through the electronic structure of the **SCNC**s.

To quantify the overall luminescence efficiencies of the nanocrystals and the Tb^{3+} metal ion, quantum yields were recorded in steady-state and time-resolved modes. The average overall quantum yield at room temperature for the nanocrystals upon excitation at 300, 305, and 310 nm was determined to be $2.5(\pm 0.1) \times 10^{-2}$. The quantum yield for the Tb^{3+}-centered emission was determined to be $3(\pm 1) \times 10^{-4}$. These relatively low quantum yield values are compensated by the high extinction coefficient of **SCNC**s and the large number of lanthanide emitters. We expect that coating the nanocrystals will increase both quantum yields. Two different types of energy transfer mechanisms can be postulated to explain sensitization of the Tb^{3+} cations: (1) Förster[19]-type of energy transfer (dipole−dipole interaction) and (2) Dexter[20]-type of energy transfer (energy transfer via electron exchange). At present, we do not have sufficient data to quantify the relative contribution of these two mechanisms to the overall energy transfer. Additional experiments are currently being done to address this question.

The time profile of Tb^{3+} luminescence was measured at room temperature. Excitation of CdSe:Tb at 354 nm in a 60/40 mixture of toluene/THF (containing 6000 ppm water, which is efficient at quenching Tb^{3+} luminescence)[7,8] shows a dual exponential decay law, with time constants of 4.7 ± 0.2 and 2.02 ± 0.06 ms. These two lifetime values are tentatively attributed to Tb^{3+} located in the interior and at the surface of the nanocrystals, respectively. As a comparison, the Tb^{3+} luminescence lifetime of a solution of $Tb(NO_3)_3$ in the presence of undoped CdSe nanocrystals has a monoexponential decay with a time constant of 1.81 ± 0.01 ms. The longest Tb^{3+} luminescence lifetime recorded for CdSe:Tb **SCNC**s is more than double this value. These results indicate that some of the Tb^{3+} cations are well protected by the **SCNC** from water quenchers and suggest that the Tb^{3+} is a dopant of the nanocrystal (surface and/or core).[18] These lifetimes are long in comparison to luminescence lifetimes commonly observed for lanthanide complexes containing organic ligands in solution.[7]

This work suggests that incorporating lanthanide cations into **SCNC**s could lead to a new family of luminescent reporters with improved photophysical properties. These preliminary results validate the proposed approach to synthesizing CdSe:Tb **SCNC**s, with the electronic structure of the nanocrystals serving as an antenna for the lanthanide cation. The long lanthanide luminescence lifetimes observed support the hypothesis that the Tb^{3+} cations are well protected from nonradiative deactivation by the **SCNC**. Since both Tb^{3+} and nanocrystal emission can be detected simultaneously, and the Tb^{3+} signal is easily discriminated through time-resolved measurements, this species has a double luminescence signature. This feature is important for biodetection agents, where a doubly verified signal helps identify false results, either negative or positive.

Acknowledgment. We would like to thank Cole VanOrmer (Department of Engineering, University of Pittsburgh) for the TEM and EDS work, Professor David H. Waldeck for his help and advice, and the Electronic, Machine, and Glass shops of the University of Pittsburgh Chemistry Department. Funding was provided through the University of Pittsburgh and partially through the National Science Foundation (Grant DBI-0352346). We dedicate this paper to the memory of Denis Mavrocordatos (1968−2003).

Supporting Information Available: Chemicals used, experimental spectroscopic procedures, and synthesis (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Petoud, S.; Cohen, S. M.; Bünzli, J.-C. G.; Raymond, K. N. *J. Am. Chem. Soc.* **2003**, *125*, 13324−13325. (b) Zhang, J.; Badger, P. D.; Geib, S.; Petoud, S. *Angew. Chem., Int. Ed.* **2005**, *44*, 2508−2512.
(2) Bünzli, J.-C. G.; Choppin, G. R. *Lanthanide Probes in Life, Chemical, and Earth Sciences: Theory and Practice*; Elsevier: Amsterdam and New York, 1989.
(3) Hemmila, I.; Webb, S. *Drug Discovery Today* **1997**, *2*, 373−381.
(4) Mathis, G. *J. Biomol. Screen* **1999**, *4*, 309−313.
(5) Sabbatini, N.; Guardigli, M.; Lehn, J. M. *Coord. Chem. Rev.* **1993**, *123*, 201−228.
(6) Weissman, S. I. *J. Chem. Phys.* **1942**, *10*, 214−217.
(7) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493−503.
(8) Supkowski, R. M.; Horrocks, W. D. *Inorg. Chim. Acta* **2002**, *340*, 44−48.
(9) Cao, Y. W. C.; Jin, R. C.; Mirkin, C. A. *Science* **2002**, *297*, 1536−1540.
(10) Rosenthal, S. J. *Nat. Biotechnol.* **2001**, *19*, 621−622.
(11) Penn, S. G.; He, L.; Natan, M. J. *Curr. Opin. Chem. Biol.* **2003**, *7*, 609−615.
(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.* **2002**, *21*, 41−46.
(13) (a) Kim, S.; Lim, Y. T.; Soltesz, E. G.; De Grand, A. M.; Lee, J.; Nakayama, A.; Parker, J. A.; Mihaljevic, T.; Laurence, R. G.; Dor, D. M.; Cohn, L. H.; Bawendi, M. G.; Frangioni, J. V. *Nat. Biotechnol.* **2004**, *22*, 93−97. (b) Chen, W.; Zhang, J. Z.; Joly, A. *J. Nanosci. Nanotechnol.* **2004**, *4*, 919−947.
(14) Bruchez, M.; Moronne, M.; Gin, P.; Weiss, S.; Alivisatos, A. P. *Science* **1998**, *281*, 2013−2016.
(15) Brukowski, T. J.; Simmons, J. H. *Cri. Rev. Solid State Mater. Sci.* **2002**, *27*, 119−142.
(16) Qu, L.; Peng, X. *J. Am. Chem. Soc.* **2002**, *124*, 2049−2055.
(17) Peng, Z. A.; Peng, X. *J. Am. Chem. Soc.* **2001**, *123*, 183−184.
(18) Raola, O. R.; Strouse, G. F. *Nano Lett.* **2002**, *2*, 1443−1447.
(19) Förster, T. *Ann. Physik*. **1948**, *2*, 55−67.
(20) Dexter, D. L. *J. Chem. Phys.* **1953**, *21*, 836−850.

JA0511725