

Eu₂O₃ particles have a useful excitation region from about 356 ~ 410 nm with a maximum at 394 nm. Another strong absorption is located at 466 nm. Following excitation at either 394 nm or 466 nm, the Eu₂O₃ particles produce an emission feature at 610 nm that was unchanged by the functionalization (Fig. 2). The emission spectrum has the following salient characteristics, typical of europium and its chelates: (1) large Stokes shift (144 nm or 216 nm, depending on excitation wavelength); (2) a narrow, symmetric emission feature at 610 nm (full width half maximum, FWHM, of 8 nm); (3) a long life time (measured with a time resolved fluorescence system to be about 300 ms).

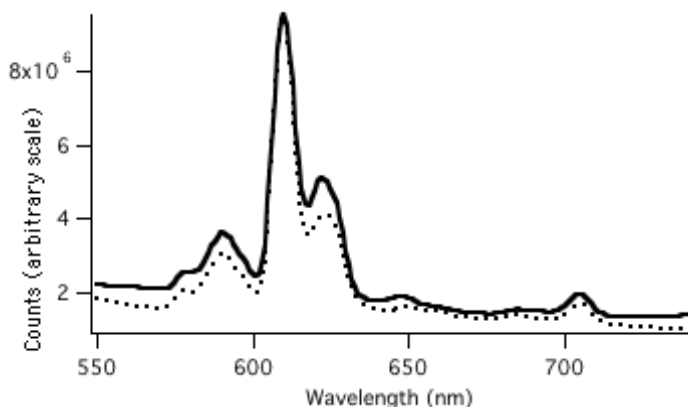


Figure 2 Emission spectra of untreated (full line) and functionalized (dashed line) Eu₂O₃ nanoparticles

To demonstrate the capability of coated Eu₂O₃ fluorescent nanoparticles as a label for biological application, we applied atrazine haptens-Eu₂O₃ particle conjugates in an immunoassay for the herbicide atrazine. The widespread use of atrazine, resulting in contaminated drinking water and food products, poses the risk of exposure for the general public (10) and for the environment (11). Recent observations of the effects of

atrazine on amphibians (12) have shown an impact on gonadal development, raising further concerns about the environmental impact of this class of compounds. Figure 3 shows fluorimeter measurements, observed at 610 nm, following the immunoassay. The detection limit of this assay is about 0.5 ng mL⁻¹. This compares with a detection limit of about 0.1 ng mL⁻¹ in a conventional ELISA (13). The sensitivity of the rapid europium-based assay is sufficient to resolve the near parts per billion (ppb) levels at which Hayes et al. (12) reported effects on the gonadal development of amphibians. The conditions for the Eu₂O₃ based assay were the same as those used in the ELISA and were not optimized in any way. By employing this new europium label, two steps in the conventional ELISA were eliminated, resulting in a higher throughput assay and a smaller coefficient of variation. The use of conventional organic fluorophores in the same assay increased speed but reduced total signal to noise over the classical ELISA due to an

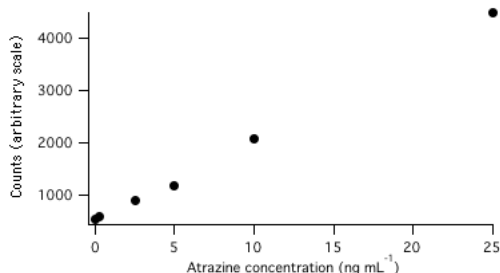


Figure 3 Emission from Eu₂O₃ used in an immunoassay for atrazine

increase in background (data not shown).

The comparison of sensitivities is very encouraging, and is indicative of the potential for the inorganic Eu nanoparticles to improve practical detection methodologies in environmental toxicology. In addition to the useful optical properties (sharp spectral features, long life time, no photobleaching), the treated particles were found to be very easily purified and conjugated to proteins or haptens. Other areas of biology may benefit from the ease of use of the functionalized

Eu₂O₃ particles. In addition, we have recently synthesized more complex crystal compositions with Eu in an alumina host that offer additional violet and green wavelengths for use in multiplexed bioassays.

Development of quantum dot materials continues. We have explored the use of near infra-red quantum dots, including PbSe and PbS. We have devised a colloidal synthesis method to form lead sulfide quantum dots. The precursors for this synthesis are powdered PbO and sulfur that are dissolved in organic surfactants. The surfactants play a dual role in that they prevent Oswald ripening during nanocrystal nucleation and growth. During the reaction, aliquots were removed to determine how the reaction time affected the crystal growth.

Lead sulfide has desirable bulk properties (a band gap of 0.41 eV and an exciton radius of 18nm), which gives it the potential to form quantum dots that will yield band gap fluorescence above 500 nm. We have observed a substantial red shift in the emission with increasing reaction time, indicating an increase in particle size.

We have also managed to synthesize a form of iron oxide nanocrystals, via laser ablation, that exhibits optical emission in the near UV and violet part of the spectrum. With magnetic properties in addition to these optical effects, this may prove to be a very useful material that can be functionalized in the manner that we have outlined above.

References

1. J. Slavik, *Fluorescent Probes in Cellular and Molecular Biology* (CRC Press, Boca Raton, FL, 1994).
2. T. Casci, *Nat. Rev. Genet.* **2**, 567 (2001).
3. W. C. W. Chan, S. M. Nie, *Sci.* **281**, 2016-2018 (1998).
4. M. Y. Han, X. H. Gao, J. Z. Su, S. Nie, *Nat. Biotechnol.* **19**, 631-635 (2001).
5. H. Mattoussi *et al.*, *Phys. Status Solidi B-Basic Res.* **224**, 277-283 (2001).
6. S. J. Rosenthal, *Nat. Biotechnol.* **19**, 621-622 (2001).
7. M. Nogami, Y. Abe, *J. Non-Cryst. Solids* **197**, 73-78 (1996).
8. A. Patra *et al.*, *J. Phys. Chem. B* **103**, 3361-3365 (1999).
9. D. Gerion *et al.*, *J. Phys. Chem. B* **105**, 8861-8871 (2001).
10. R. Loosli, *Rev. Environ. Contam. Toxicol.* **143**, 47-57 (1995).
11. R. Renner, *Env. Sci. Tech.* **36**, 55A-56A (2002).
12. T. Hayes *et al.*, *Nature* **419**, 895 - 896 (2002).
13. M. Wortberg, S. B. Kreissig, G. Jones, D. M. Rocke, B. D. Hammock, *Anal. Chim. Acta* **304**, 339-352 (1995).