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Application of Quantum Dots to Environmental and Cell Biology

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Fluorescent labeling of molecules is a standard technique in biology (*1*). The labels are often organic dyes that give rise to the usual problems of broad spectral features, short life time, photobleaching, and potential toxicity to cells. Recently, nanoparticles such as quantum dots have attracted a great deal of attention from the biology community because of the advantages they offer over conventional dyes (*2-6*). These particles can have a strong optical emission that exhibits a spectral peak that is sharper than typical organic dyes, with a large Stokes shift, and with minimal influence by other chemicals. In addition to semiconductor quantum dots, simple inorganic phosphors such as Eu_2O_3 have been recognized as offering potential in obtaining high emission intensity (*7, 8*). Unfortunately, natural or untreated Eu_2O_3 particles are insoluble in water and are easily dissolved by acid during activation and conjugation, losing their desirable optical properties. Coating the particles can protect the particle from being dissolved by acid, and may provide useful functional groups for biological conjugation.

Chelated lanthanide labels offer the potentially significant advantage of a relatively long-lived emission, if quenching by water and dissolved oxygen can be avoided. In time-resolved (or time-gated detection) fluorometric assays with europium chelate labels, the background can be substantially reduced by detecting the signal after a time delay – in this way, the short-lived, nonspecific background interference is effectively reduced. However, the chelation chemistry is often expensive and complex. As a result, their application has been limited.

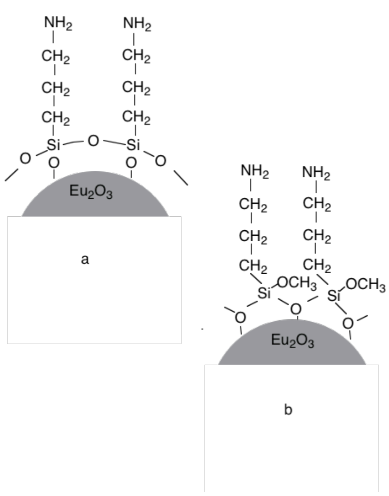

Figure 1 Structure of functional layer on Eu_2O_3

We have developed a new way for using simple europium oxide labels that provide an amino group for conjugation by the use of a microwave process. We found that 3-aminopropyltrimethoxysilane (APTMS) is microwave transparent, whereas Eu_2O_3 absorbs microwave energy, as a result of either an intrinsic property, or more likely, as a result of water or OH attached to the particle surfaces. As a result of this differential absorption in the particle suspension, heating can be confined to local regions without the need to heat the bulk solution. Reaction between the APTMS and the particle is concentrated at the particle surface. A reaction at the solid-liquid interface yields a layer of $Si\text{-}O\text{-}(CH)_x\text{-}NH_2$ that is covalently bound to the Eu_2O_3 nanoparticles. Two possible arrangements are shown in Fig. 1, presenting a structure that is analogous to that proposed by Gerion et al. (*9*) who developed a complex wet chemical method for functionalizing CdSe/ZnS quantum dots with a similar capping layer.

Eu_2O_3 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 Eu_2O_3 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).

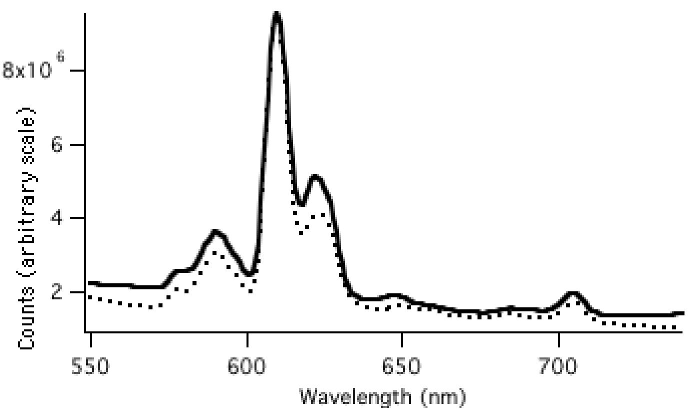

Figure 2 Emission spectra of untreated (full line) and functionalized (dashed line) Eu_2O_3 nanoparticles

To demonstrate the capability of coated Eu_2O_3 fluorescent nanoparticles as a label for biological application, we applied atrazine hapten-Eu_2O_3 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 Eu_2O_3 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 increase in background (data not shown).

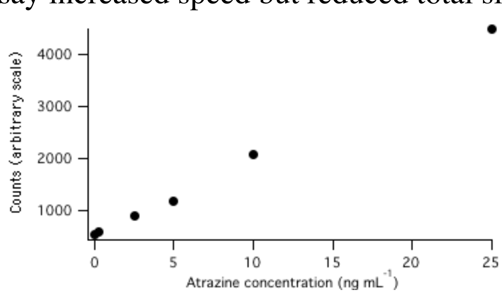

Figure 3 Emission from Eu_2O_3 used in an immunoassay for atrazine

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

Eu_2O_3 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.

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