A Novel Efficient FRET System: $CePO_4:Tb^{3+}$ Nanocrystal as Donor and Rhodamine B Dye as Accepter

Lun Wang,* Binbin Qian, Hongqi Chen, Yan Liu, and Ani Liang
Anhui Key Laboratory of Chemo-Biosensing, College of Chemistry and Materials Science, Anhui Normal University, Wuhu 241000, P. R. China

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By using a facile, wet-chemical approach, luminescent nanocrystals were prepared from nitrate and sodium tripolyphosphate precursors. These nanocrystals were stable and have extremely narrow emission bands and high dispersiblility. A novel fluorescence resonance energy-transfer (FRET) system with $CePO_4:Tb^{3+}$ nanocrystals as energy donor and Rhodamine B (RhB) dye as energy accepter was developed. The results showed that the FRET process occurred between $CePO_4:Tb^{3+}$ nanocrystal donor and Rhodamine B acceptor efficiently.

The use of Förster, or fluorescence resonance energy transfer (FRET), as a spectroscopic technique has been in practice in various areas, such as structural elucidation of biological molecules and their interactions, in vitro assays, in vivo monitoring in cellular research, nucleic acid analysis, signal transduction, and light harvesting.[1–5] But a major challenge is the design of an efficient FRET system, because the rate of energy transfer is highly dependent on many factors, such as the extent of spectral overlap, the relative orientation of the transition dipoles, and, most importantly, the distance between the donor and acceptor molecules.[6]

In recent years, rare-earth phosphate nanocrystals have attracted growing interest for use as luminescent biolabels owing to their properties, which include long lifetimes, high quantum yields (up to 61%),[7] low photobleaching, expected low toxicity, and high chemical stability.[8–13] Long-lifetime donors (fluorescent lifetime $t > 100$ ns to several ms) have a number of technical advantages over conventional fluorescent dyes ($t = 1$–5 ns). The principal benefit arises from the ability, through time-resolved measurements, to eliminate background fluorescence (from direct excitation of dyes, scattering, and autofluorescence from cells and biomolecules), thereby dramatically improving sensitivity. Lanthanide probes also possess many distinctions, sharp emission bands, and large Stokes shifts, so that D/A emission can be detected far from the excitation wavelength. Together these properties allow lanthanide probes to be coupled to a wide range of acceptor dyes. Rhodamine derivatives, which are usually used as an acceptor in FRET systems, are prized for their great photostability, pH insensitivity over a broad range (low to neutral pH), and the ability of their fluorescence characteristics to be tailored for a particular application.[14] As we know that the donor and acceptor of a FRET system should be functionalized to improve the differential reciprocity,[15–18] which shortens the distance between the donor and acceptor molecules. Then, an efficient FRET system can be built. In this paper, stable, highly, dispersible, and unfunctionalized $CePO_4:Tb^{3+}$ nanocrystals were prepared. The reaction between the nanocrystals and Rhodamine B was studied. We found that the efficiency of the energy transfer between them with $CePO_4:Tb^{3+}$ nanocrystals can be up to 50% which indicated that an efficient FRET process occurred between them with $CePO_4:Tb^{3+}$ nanocrystal as donor and Rhodamine B (RhB) as acceptor.[19] $CePO_4:Tb^{3+}$ nanocrystals were prepared by using facile, solvothermal technology. $Ce(NO_3)_3$ (4.5 mL, 0.1 mol L^{-1}), $Tb(NO_3)_3$ (0.5 mL, 0.1 mol L^{-1}), and sodium tripolyphosphate (TPP, 10 mL, 0.1 mol L^{-1}) were added to a mixture of β-cyclodextrin (5 mL, 0.01 mol L^{-1}) and water (10 mL), and the solution was thoroughly stirred. Subsequently, the milky colloidal solution was transferred to a Teflon-lined stainless-steel autoclave with a capacity of 30 mL and heated at 90 °C for 3 h. The system was then allowed to cool to room temperature. The final product was collected by means of centrifugation, washed once with ethanol and twice with deionized water to remove any possible remnants, and then dried in air. Dilute colloidal solutions (5 g L^{-1}) were obtained by redispersing the nanocrystals in water.

Lanthanide ions have very low extinction coefficients ($\approx 1\ M^{-1}\ cm^{-1}$), which make them difficult to excite directly, so a light-harvesting antenna or sensitizer is required. The sensitizer molecule absorbs incident light and transfers this energy to the lanthanide ion. It is well known that Ce^{3+} can enhance the green emission of terbium. Figure 1a is the fluorescence spectra of $CePO_4:Tb^{3+}$ nanocrystals (ex = 275 nm) which originates from their bulk property: transitions between d and f electron states and their local symmetry. This result indicates that an effectual intermolecular energy transfer from Ce^{3+} to Tb^{3+} occurs in the $CePO_4:Tb^{3+}$ nanocrystals, as observed for the bulk power material.[20]

The FRET process resulting from dipole–dipole interactions is strongly dependent on the center-to-center separation distance and it requires a nonzero integer of the spectral overlap between donor emission and acceptor absorption.[21] UV–vis absorption spectra of RhB showed a peak at 552 nm (Figure 1b). A strong emission of $CePO_4:Tb^{3+}$ could be seen as a narrow band between 530 and 570 nm. The degree of the overlap was great

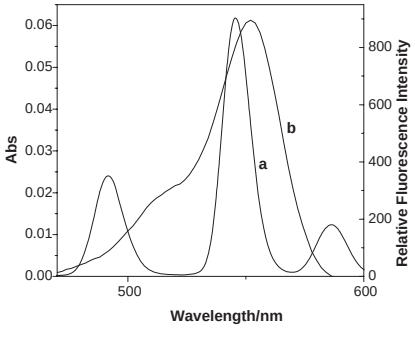

Figure 1. a) The fluorescence emission spectra of the $CePO_4:Tb^{3+}$ nanocrystals. b) The absorption spectra of RhB.

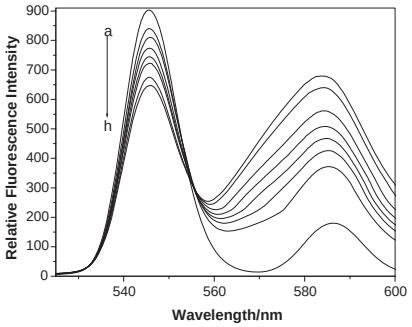

Figure 2. The fluorescence emission spectra of the $CePO_4$:Tb^{3+}–RhB system. Concentration of Rhodamine B: (a) 0.00 mol L^{-1}, (b) 0.6×10^{-6} mol L^{-1}, (c) 0.8×10^{-6} mol L^{-1}, (d) 1.0×10^{-6} mol L^{-1}, (e) 1.2×10^{-6} mol L^{-1}, (f) 1.4×10^{-6} mol L^{-1}, (g) 1.6×10^{-6} mol L^{-1}, (h) 2.0×10^{-6} mol L^{-1}, $CePO_4$:Tb^{3+} nanocrystals: 0.1 g L^{-1}. pH: 5.00; At room temperature.

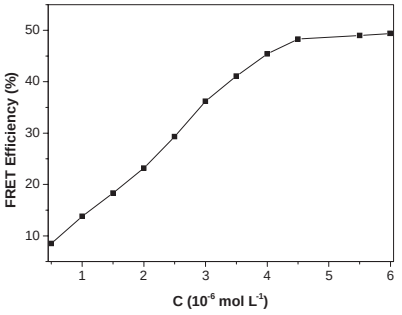

Figure 3. The effect of the concentration of RhB on the efficiency of the energy transfer between $CePO_4$:Tb^{3+} nanocrystals and RhB.

between the emission spectrum of $CePO_4$:Tb^{3+} and the absorption spectrum of RhB.

Fixing the amount of $CePO_4$:Tb^{3+}, the quantity of RhB was gradually increased to observe the change of the fluorescence spectra of $CePO_4$:Tb^{3+}/RhB complex solution. In this case, an efficient FRET system has been built between the $CePO_4$:Tb^{3+} and RhB. The fluorescence intensity of $CePO_4$:Tb^{3+} nanocrystals at 546 nm gradually decreased, meanwhile, while that of RhB was enhanced (Figure 2). The results indicate that the FRET process occurred between $CePO_4$:Tb^{3+} nanocrystal donor and RhB acceptor efficiently. In a separate experiment, the effect of the concentration of RhB on the efficiency of the energy transfer was studied in detail (Figure 3). It lies above 20% for a wide range of the concentration of RhB varied between 2 and 6×10^{-6} mol L^{-1}. Considering the efficiency of the transfer as a function of the concentration of RhB, it increases from 8.5% and finally reached a maximum value 50% when the concentration of RhB is increased from 0.5 to 4.5×10^{-6} mol L^{-1}. It then saturates and the change of the efficiency of the transfer was not obvious when the concentration of RhB is further increased from 4.5 to 6×10^{-6} mol L^{-1}. The increase followed by the saturation of the energy-transfer efficiency with increasing number of acceptors is typical of FRET.[19] As the number of acceptors is increased, the probability of energy transfer from a donor to an acceptor is expected to become larger until it reaches saturation.[22] The effecting factors on the fluorescence intensity of the FRET system, have been studied in detail (See Supporting Information).[23] The results show that the interaction of $CePO_4$:Tb^{3+} and RhB is mainly the result of nonelectrostatic interaction and that fluorescence quenching is partially attributed to dynamic quenching. The effect of the concentration of $CePO_4$:Tb^{3+} on the UV spectra of the FRET system indicates that no reaction between $CePO_4$:Tb^{3+} nanocrystals and RhB dye happened.[23]

In summary, $CePO_4$:Tb^{3+} nanocrystals were prepared by using a facile, solvothermal strategy. These nanocrystals were stable, high-quality, dispersible and unfunctionalizing. With $CePO_4$:Tb^{3+} nanocrystal as donor and RhB dye as accepter, an efficient FRET system was built. Factors effecting on the system have been studied in detail. The system can be used to study the structural elucidation of biological molecules and their interactions when there is no differential reciprocity between the biological molecules. Furthermore, these luminescent nanocrystals are also potential fluorescent biolabels for use in other biological and clinical applications, such as in fluorescence imaging and for immunoassays.[24,25]

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