ORGANIC LETTERS

2011
Vol. 13, No. 11
2802–2805

Intermolecular Energy Transfer from Tb^{3+} to Eu^{3+} in Aqueous Aggregates and on the Surface of Human Cells

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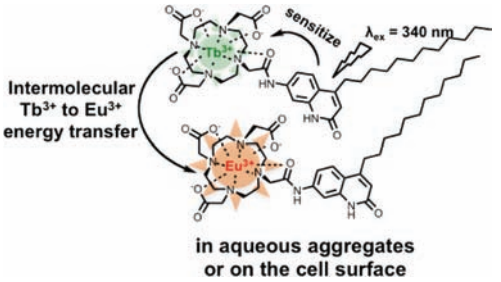
Received February 9, 2011

ABSTRACT



Efficient intermolecular energy transfer from carbostyril 124-sensitized Tb^{3+} to Eu^{3+} in aqueous aggregates is reported. This energy transfer was also recapitulated on the cell surface of a human kidney cell line (HEK-293T) and imaged by fluorescence microscopy as an example for the applicability of this energy transfer probe for imaging in biological systems.

Fluorescence resonance energy transfer (FRET) and luminescence resonance energy transfer (LRET) are powerful tools for studying molecular interactions in solution and in a cellular context.[1] Extensive research has been carried out using resonance energy transfer between various energy transfer partners, such as small organic fluorophores,[2] fluorescent proteins,[3] quantum dots,[4] and fluorescent/luminescent metal ions.[5,6] In particular, long-lived luminescent lanthanide ions as energy transfer donors[6] have been shown to offer many advantages, since light output can be measured in a time domain that is essentially background-free.[7] The use of a second lanthanide ion as an energy transfer acceptor can make the resonance energy transfer measurement more sensitive because the emissions from a donor and an acceptor are readily resolvable as sharply spiked peaks. Energy transfer between two lanthanide ions in heterodinuclear complexes and mixed lanthanide systems has long been known in

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10.1021/ol200328p
Published on Web 05/12/2011

solution[8] and in the solid state.[9] However, there are rare examples of *intermolecular* energy transfer between Tb^{3+} and Eu^{3+} in aqueous solution,[10] as well as its application for biological sensing or cellular imaging.[11] As a part of a broad program aimed at the development of optical probes for imaging metabolic and signaling events in cells and tissues,[12] we became interested in exploring energy transfer between lanthanide(III) ions and its applicability. Herein, we report intermolecular energy transfer from carbostyril 124-sensitized Tb^{3+} to Eu^{3+} in aqueous aggregates and show the first example of imaging this phenomenon on the cellular membrane of HEK-293T cells.

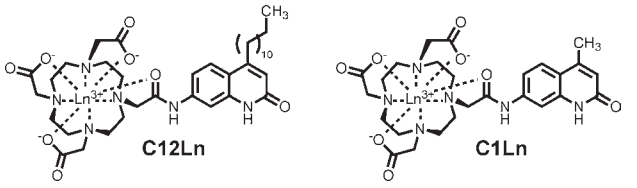
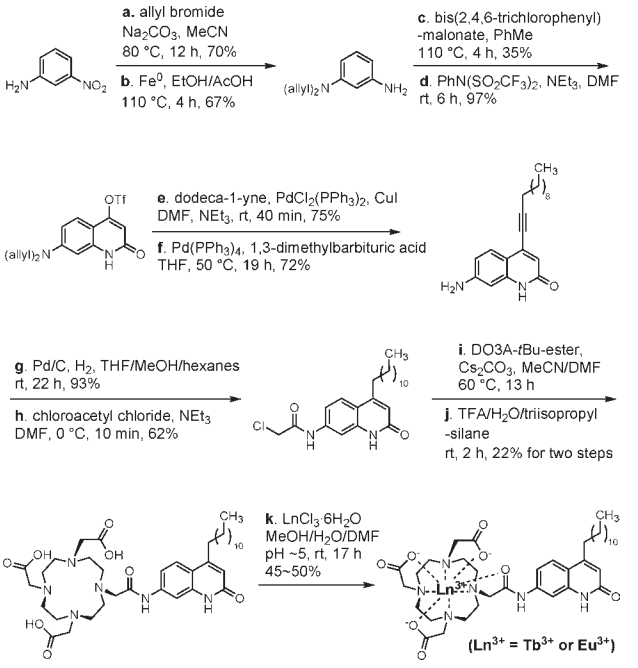


Figure 1. Structure of C12Ln and C1Ln (Ln = Tb or Eu).

Two sets of lanthanide complexes, C12Ln and C1Ln (Ln = Tb or Eu, Figure 1), were prepared after 11- and 4-step sequences from 3-nitroaniline (Scheme 1) and 7-amino-4-methylquinolin-2(1*H*)-one (see Supporting Information), respectively, followed by purification by preparative reversed-phase HPLC. The amphiphilic C12Ln with a polar Ln:DOTA (1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) head and a nonpolar dodecane chain was designed to have intermolecular proximity in water by forming micelle-like aggregates. On the other hand, the control compound, C1Ln, does not form aggregates in aqueous solution but exists as free solutes. 4-Methylcarbostyril (Cs124) was chosen as the organic photon antenna because it is known to sensitize terbium luminescence much more efficiently than europium luminescence,[13] so the energy transfer from terbium to europium can be readily observed as quenched terbium emission and enhanced europium emission with minimum background.

To examine the potential of C12Ln to form aggregates in aqueous solution, the concentration dependence of time-resolved emission profiles of C12Tb and C1Tb was examined (Figure 2A and 2B). Luminescence decay curves of

Scheme 1. Synthesis of C12Ln



C12Tb from 2 to 40 μM are biexponential, indicating that C12Tb exists as two distinct species with different lifetimes (1.31 ± 0.06 and 0.27 ± 0.04 ms) in aqueous solutions, presumably monomeric C12Tb with a longer lifetime and C12Tb aggregates with a shorter lifetime mainly due to

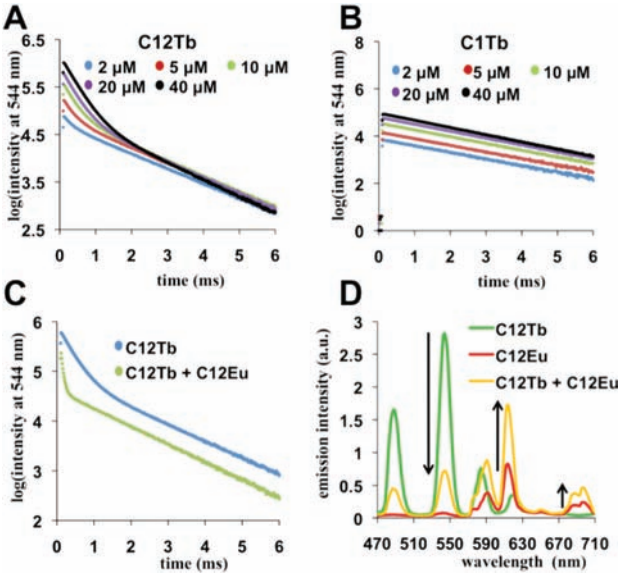


Figure 2. Tb^{3+} luminescence decay curves of (A) C12Tb and (B) C1Tb at different concentrations (2, 5, 10, 20, and 40 μM); (C) 20 μM C12Tb and a cocktail of 20 μM C12Tb + 20 μM C12Eu (in H_2O, λ_{ex} = 340 nm, λ_{em} = 544 nm). (D) Steady-state luminescence spectra of 20 μM C12Tb, 20 μM C12Eu, and a cocktail of 20 μM C12Tb + 20 μM C12Eu (in H_2O, λ_{ex} = 340 nm).

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self-quenching.[14] Indeed, the composition of these two species changes in a direction to favor shorter lifetime species (from 8% to 75%, see Supporting Information, Figure S2) as the concentration increases from 2 to 40 μM. In contrast, C1Tb exhibits monoexponential emission decay curves with a luminescence lifetime of 1.56 $\pm$ 0.02 ms regardless of the concentration, indicating its homogeneous distribution in water. Addition of 20 μM C12Eu to an aqueous solution of 20 μM C12Tb quenched terbium emission selectively from the short-lived, presumably aggregated component, while not affecting the lifetime of the long-lived component (Figure 2C). Moreover, the heterometallic cocktail of C12Tb and C12Eu exhibits significantly decreased emission from terbium, whereas the emission from europium enhances by 2-fold at 700 nm (Figure 2D). These results together suggest that energy transfer takes place from Cs124-sensitized terbium to europium when the two complexes are brought into proximity by forming mixed aggregates in aqueous solution (Figure 3).

The efficiency of energy transfer was investigated as a function of solvent polarity. When the emission was monitored at 544 nm, the magnitude of the reduction in

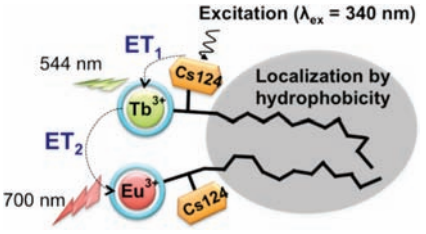

Figure 3. Schematic representation of intermolecular energy transfer from Cs124-sensitized Tb^{3+} to Eu^{3+} in amphiphilic C12Tb + C12Eu cocktail.

terbium emission resulting from the presence of C12Eu decreased as DMSO content was increased (Figure 4A, blue diamonds). A similar polarity effect was also observed on the europium emission at 700 nm (Figure 4B, blue diamonds) to give a smaller difference in emission intensities between C12Eu and the cocktail at lower polarity. This implies that the energy transfer efficiency is solvent-polarity-dependent and that it is stronger in pure water than in solvent mixtures of lower polarity, presumably because the propensity for the C12Ln complexes to form aggregates in water by hydrophobic interaction is driven by solvent polarity. In contrast to C12Ln, emissions at 544 and 700 nm from C1Tb and C1Eu, respectively, are not affected by each other's copresence at varying solvent polarity (Figure 4A and B, red triangles), suggesting their existence

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(15) The bimolecular energy transfer rate constant from C1Tb to C1Eu in H_2O was determined to be 3×10^6 M^{-1} s^{-1} which is more than 3 orders of magnitude smaller than the diffusion limit (for more details, see Supporting Information, Figure S3).

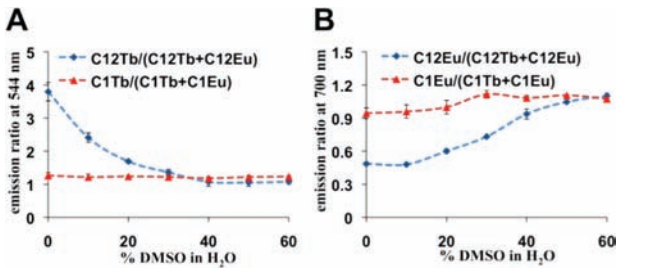

Figure 4. Emission ratio of (A) C12Tb and a cocktail of C12Tb + C12Eu (blue diamond), C1Tb and a cocktail of C1Tb + C1Eu (red triangle) at 544 nm; (B) C12Eu and a cocktail of C12Tb + C12Eu (blue diamond), C1Eu and a cocktail of C1Tb + C1Eu (red triangle) at 700 nm in water with increasing % (v/v) of DMSO. [C12Ln] or [C1Ln] = 20 μM[16] in all measurements. Error bars: s.d. (n = 3).

as free solutes in aqueous solution and the low efficiency of energy transfer by simple diffusional collisions.[15]

Intermolecular energy transfer from Tb^{3+} to Eu^{3+} was recapitulated on the cell surface and imaged by fluorescence microscopy as a preliminary study for bioimaging applications. As the dodecane hydrocarbons are reported to be sufficiently lipid-like,[17] the lipophilic chains of C12Ln should guide the compounds to be inserted into the cellular

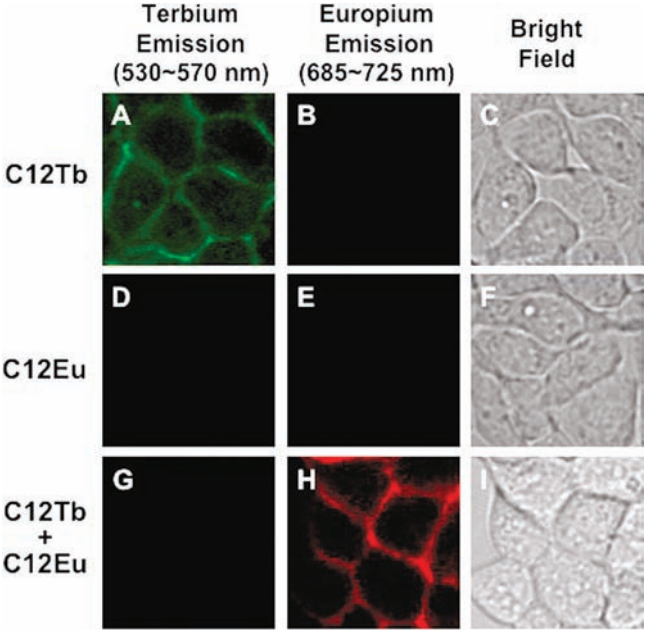

Figure 5. Luminescence and bright field images of HEK-293T cells incubated with 20 μM C12Tb (A–C), 20 μM C12Eu (D–F), and a cocktail of 20 μM C12Tb + 20 μM C12Eu (G–I) for 15 min. Emissions from terbium and europium were imaged using a fluorescence microscope mounted with two filter sets, one with a 350 $\pm$ 25 nm exciter and 550 $\pm$ 20 nm emitter (for terbium emission) and the other with a 350 $\pm$ 25 nm exciter and 705 $\pm$ 20 nm emitter (for europium emission).

plasma membrane. When HEK-293T cells were treated with 20 μM C12Tb for 15 min at 37 °C, a luminescence image of the cell surface was observed (350 ± 25 nm excitation and 550 ± 20 nm emission, Figure 5A) which indicates that the cellular plasma membrane was selectively labeled with C12Tb. In contrast, cells incubated with 20 μM C12Eu did not afford any detectable luminescence signals (350 ± 25 nm excitation and 705 ± 20 nm emission, Figure 5E) because of the inability of Cs124 to efficiently sensitize europium. Importantly, upon incubating the cells with a cocktail of 20 μM C12Tb and 20 μM C12Eu, strikingly enhanced Eu^{3+} luminescence (Figure 5H) and correspondingly quenched Tb^{3+} luminescence (Figure 5G) were observed, demonstrating the energy transfer taking place on the cell surface from Cs124-sensitized terbium to europium. This represents the first example of cellular imaging based on intermolecular energy transfer between two lanthanide ions, which could be the basis for novel optical sensors that extend the scope of FRET/LRET-based techniques to include the measurement of two long-lived emission signals.

In summary, we present support for the intermolecular energy transfer from Cs124-sensitized Tb^{3+} to Eu^{3+} in aqueous aggregates as shown by steady-state and time-resolved luminescence studies and demonstrate that this effect is sensitive to solvent polarity. We also image this phenomenon on the cell surface, suggesting its potential use for the development of novel optical biosensors.

Acknowledgment. Financial support for this work was provided by the G. Harold & Leila Y. Mathers Foundation. S.J. and N.J.T. thank the National Science Foundation for financial support through Grant NSF-CHE-07-17518. We thank Dr. Julien Massue (University of Strasbourg, France) for his intellectual and editorial support.

Supporting Information Available. Detailed synthetic procedures, characterization of new compounds, experimental details for photophysical studies, and fluorescence microscope imaging protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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