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Luminescent Lanthanide Complexes with Analyte-Triggered Antenna Formation

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Supporting Information

ABSTRACT: A new strategy for accessing analyte-responsive luminescent probes is presented. The lanthanide luminescence of Eu and Tb centers is switched on by the analyte-triggered formation of a sensitizing antenna from a nonsensitizing caged precursor. As the cage can be freely varied, an array of probes for different analytes ($Pd^{0/2+}$, H_2O_2, F^-, β-galactosidase) can be created from the same core structure. The probe design affords nanomolar to micromolar detection limits, provides the capability to detect two analytes in parallel, and can be utilized to monitor enzymatic activity in live cells.

Fluorescence spectroscopy is a sensitive, minimally invasive technique for the monitoring of biologically relevant species and processes and provides a simple, low-cost alternative to established techniques (e.g., atomic absorption/emission spectroscopy) for the detection of environmental contaminants. Responsive probes based on luminescent lanthanides (Ln), which combine superior emission properties[1] with versatility, are made possible by placing Ln sensitization by the light-harvesting antenna under analyte control.[2] Ln probes to measure citrate and lactate in biological fluids and pH, $^1O_2$, and NO in cells have been reported.[3] Despite these successes, the development of new responsive Ln probes is still a major undertaking. The current strategies for creating new probes are often tedious and/or are applicable to only a single analyte without the possibility of extension to additional ones.[2,4] The narrow, nonoverlapping Ln emissions enable ratiometric imaging with mixtures of Eu and Tb complexes[5] and the detection of multiple luminescent labels.[6] However, to the best of our knowledge, the simultaneous detection of two analytes with responsive Ln probes has not been reported, possibly reflecting the difficulties of constructing two noninterfering probes using established methods.

Here we present a Ln probe design that (1) is simple to synthesize, (2) gives a turn-on emission over zero background, (3) is selective for a single analyte over potentially competing ones, and (4) can easily be adapted to detect a wide array of different analytes by only a predictable modification of the core structure. This design yields significant improvements over previously reported H_2O_2-responsive Ln probes and has enabled the creation of new Ln probes for analytes for which only organic-fluorophore-based probes have been reported ($Pd^{0/2+}$, F^-, β-galactosidase). In addition to being valid targets in their own right because of their environmental and biological importance,[7] these species also cover an unprecedented breadth of analyte types.

We envisioned that a nonsensitizing antenna precursor such as the caged coumarin in **Ln1** could be built into a Ln complex framework (Figure 1). Uncaging by the analyte triggers a reaction that forms the sensitizing antenna in situ (a coumarin in **Ln2**; Figure 1). As sensitization is dependent on a chemoselective reaction as opposed to a binding event, the development of Ln probes for species that are difficult to detect with supramolecular probes (e.g., several transition metals,

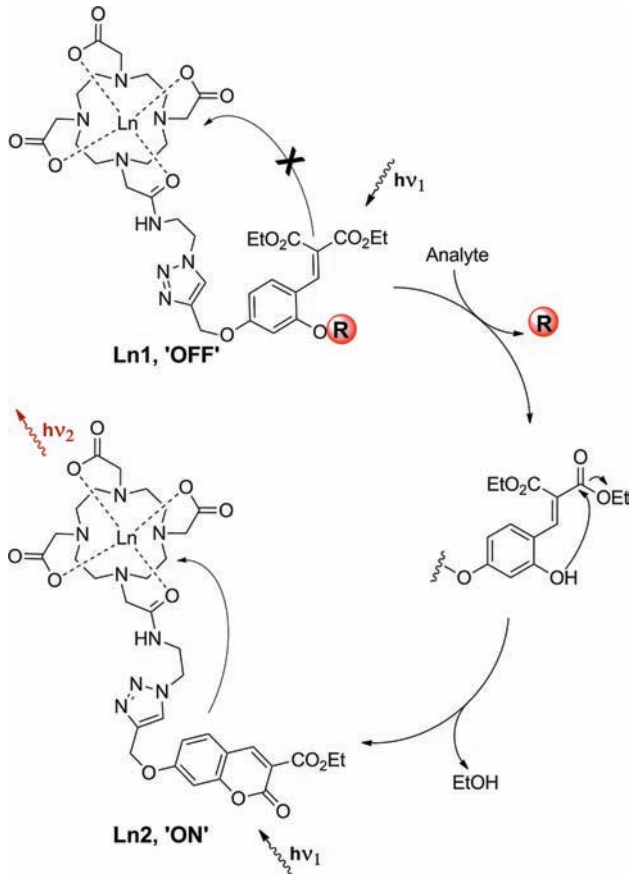

Figure 1. Analyte-triggered antenna formation.

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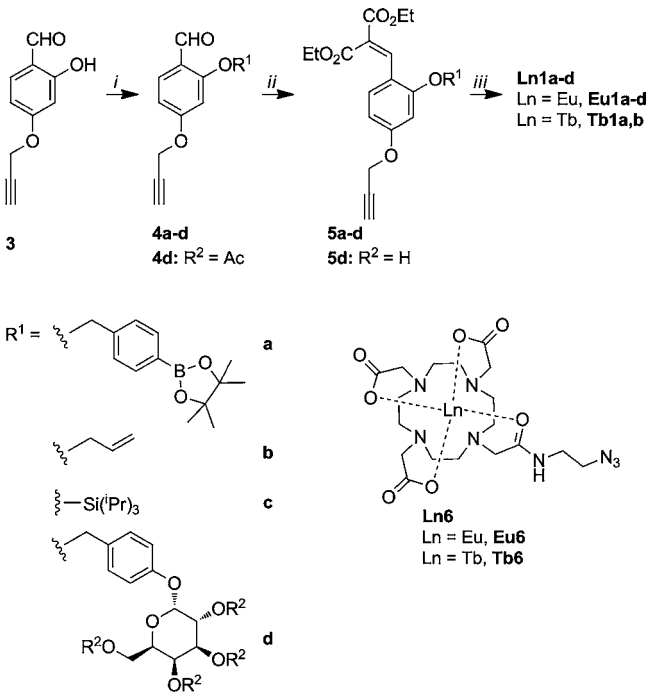
neutral molecules) is possible.[8] The same architecture can carry different caging groups; thus, a single molecular design can be used for diverse analytes. Coupling this with coumarins, which are known sensitizers of both Tb and Eu,[9] further enhances the application of the design by enabling the independent monitoring of two analytes with two probes that possess two different caging groups. Analyte-mediated introduction of the antenna to obtain a turn-on response over zero background has been used before to detect Cu(I)[4a] and $OH^{\bullet}$.[4d] However, the detection mechanisms of those probes are restricted to their respective analytes. Furthermore, their bimolecular nature precludes intracellular applications, as this would require efficient colocalization of the Ln complex, antenna, and analyte.

Benzylboronic acid-caged[10] **Ln1a** (detects H_2O_2), allyl-caged[11] **Ln1b** (detects $Pd^{0/2+}$), TIPS-caged[12] **Eu1c** (detects F^-), and galactose-caged **Eu1d** (detects β-galactosidase) were synthesized by the short route shown in Scheme 1. Caging

Scheme 1. Synthesis of Ln Probes[a]



[a]Conditions: (i) R−Br, K_2CO_3, CH_3CN (**4a**, **4b**, **4d**) or TIPS-Cl, DBU, CH_2Cl_2 (**4c**); (ii) diethyl malonate, piperidine (cat.), 3 Å molecular sieves, CH_3CN (then for **4d**, K_2CO_3, MeOH); (iii) $CuSO_4$, NaAsc, TBTA, 2:1:1 H_2O/^{t}BuOH/THF.

groups were attached either directly to the phenolic oxygen or via a 4-benzyloxy linker; in the latter case, initial uncaging was followed by quinone−methide[13] elimination [Scheme S1 in the Supporting Information (SI)]. Aldehyde **3**[14] was O-alkylated with the required bromides or silylated with TIPS-Cl, yielding **4a−d**, which in turn were treated with excess diethyl malonate in the presence of 3 Å molecular sieves and catalytic piperidine to afford **5a−d** in good to excellent yields. Importantly, the caged antennae **5a−d** were combined with **Ln6** in the final step in a Cu(I)-catalyzed cycloaddition.[15] This reaction is tolerant of caging groups that could succumb to the harsh conditions traditionally employed for ligand synthesis and complexation (i.e., acidic/basic ester hydrolysis, prolonged heating with Ln salts).

Caged **Ln1a−d** were essentially nonemissive when excited at 356 nm, while **Eu2** and **Tb2** displayed intense Ln-centered luminescence (>80-fold larger emission at 595 nm for **Eu2** relative to **Eu1b**; Φ_{Eu2} = 0.31%). Exposure of **Ln1a−d** to their respective analytes switched on the Ln emission (Figures S1−S5 in the SI). The identity of the emissive product was confirmed to be **Ln2** by high-resolution electrospray ionization mass spectrometry and UV−vis and fluorescence spectroscopy. Uncaging and coumarin formation were seen when the **5a−c** → **7** reaction (Scheme 2) was followed by ^{1}H NMR analysis

Scheme 2

(Figures S6 and S7). The number of Ln-bound water molecules in **Eu2** was measured[16] to be ∼1 (Table S1 in the SI), in accordance with the eight-coordinate macrocyclic ligand structure.

We investigated the analyte-triggered reactions **Ln1a−d** → **Ln2**. Time-resolved luminescence titrations of **Eu1a** and **Tb1a** showed that the probe response was linear in the 0−200 μM [H_2O_2] range. H_2O_2 concentrations as low as 1 μM could be detected at pH 7 at ambient temperature (Figure 2), which

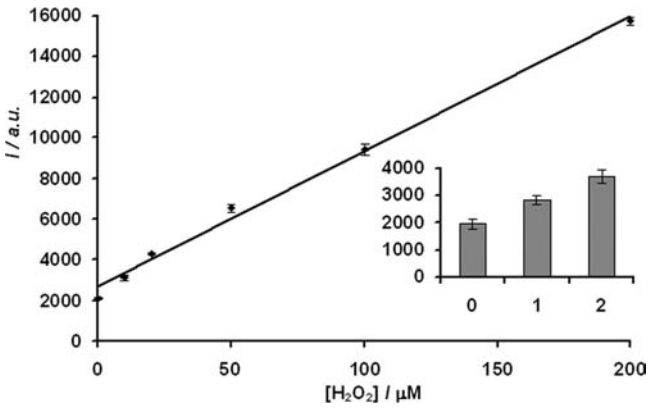


Figure 2. Time-resolved luminescence titration of **Eu1a** with H_2O_2 (10 μM **Eu1a**, pH 7 HEPES buffer, 1 h, r.t., λ_{ex} = 356 nm, λ_{em} = 615 nm, 50 μs delay, 1050 μs sample window). Inset: Eu luminescence at [H_2O_2] = 0, 1, and 2 μM (1.5 h, r.t., λ_{em} = 594 nm).

bodes well for potential biological applications. H_2O_2 has been implicated in the emergence of pathologies such as cancer and neurodegenerative diseases and is also a mediator of signal transduction, making its detection at low micromolar concentrations an important goal.[7a,b,17] The performance of **Eu1a** is comparable to those of the best organic-chromophore-based fluorescent probes[18] and superior to those of known Ln-based H_2O_2 probes, with higher sensitivity and a larger turn-on response (>30-fold, 30 min, 1 mM H_2O_2).[19]

We found that **Eu1b** could detect Pd^0 [using $Pd(PPh_3)_4$ as a source] and Pd^{2+} in the presence of PPh_3 (Figure 3a and Figure S8). **Eu1b** was selective for Pd^0 over a range of metal cations (Figure 3b), with only π-philic[20] Pt^{2+} reacting with **Eu1b** (∼18% of the response observed with Pd^0). Detection of Pd in the presence of these cations revealed that only Au^{3+} interfered with **Eu2** formation (Figure S9), likely because Au^{3+} is a strong oxidant.[21]

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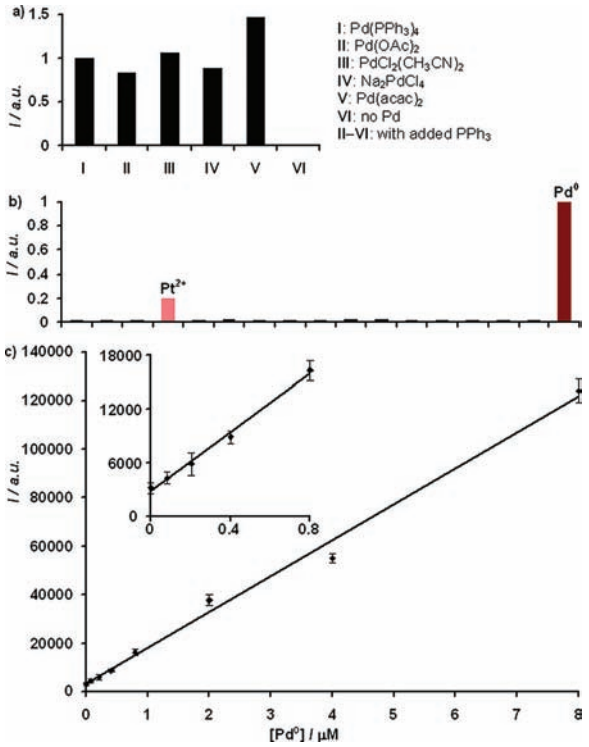

Figure 3. (a) Evaluation of Pd sources. (b) Selectivity of **Eu1b** for Pd^0 over metal cations (Zn^{2+}, Ru^{3+}, Rh^+, Pt^{2+}, Ni^{2+}, Mn^{2+}, Mg^{2+}, Li^+, K^+, Fe^{3+}, Fe^{2+}, Cu^{2+}, Co^{2+}, Au^{3+}, Au^+, and Ag^+ left to right). (c) Titration curve for **Eu1b** with Pd^0 (10 μM **Eu1b**, CH_3CN:pH 10 borate buffer (1:1), 10 min, r.t., λ_{ex} = 356 nm, λ_{em} = 699 nm).

Fluorescence titration of **Eu1b** with $Pd(OAc)_2/PPh_3$ showed that [Pd] as low as 80 nM elicited Eu emission within 10 min at ambient temperature, with a linear response in the 0−8 μM range (Figure 3c). We expect that this remarkable performance, which is comparable to those of the best organic-chromophore-based probes,[11,22] could be further improved by additional optimization of the detection conditions. Pd is a known environmental contaminant and Pd-catalyzed reactions are popular for drug synthesis, making Pd detection of considerable interest.[7c,d]

In the presence of fluoride ions, **Eu1c** provided an increase in Eu emission that was concentration-dependent over the 0−200 μM range, with a detection limit of ∼2 μM in DMSO (Figure S10). Taken together, these results show that our probe design can be readily applied to the sensitive detection of a broad variety of small-molecular weight species.

Analyte-triggered antenna formation also enables the development of turn-on Ln probes for enzymatic activity. Exposure of **Eu1d** to purified β-galactosidase yielded intense Eu emission, a result that was reproduced in *lacZ*+ bacterial cell lysates but not the control *lacZ*− bacterial cell lysates (Figures S11 and S12). More impressively, β-galactosidase activity could be monitored in real time during live cell growth. The addition of 10 μM **Eu1d** to the culture medium of *lacZ*+ bacteria constitutively expressing β-galactosidase, but not the addition to *lacZ*− bacteria, resulted in an intense Eu-centered emission within ∼5 min that increased until reaching a plateau at ∼90 min (Figure 4). Having established that the time scale of **Eu1d** uptake and turnover was ∼5 min, it was reasonable to interpret the gradual increase in Eu emission as a sign of de novo β-galactosidase synthesis. These compounds were apparently

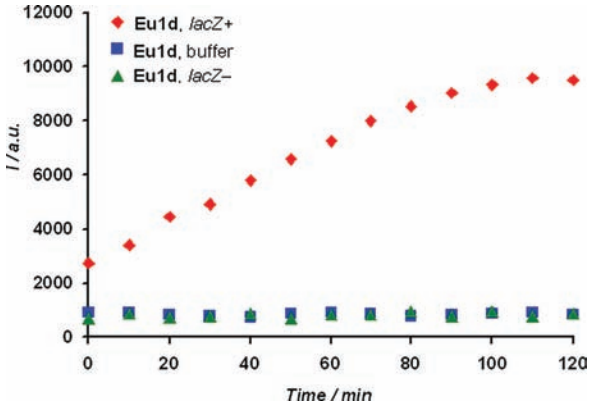

Figure 4. Monitoring of de novo β-galactosidase synthesis with **Eu1d** (10 μM **Eu1d**, λ_{ex} = 356 nm, λ_{em} = 590 nm, 37 °C).

nontoxic to the cells as well, since neither **Eu2** nor **Eu1d** interfered with bacterial growth up to 25 μM (Figure S13). These results highlight the potential application of these compounds for monitoring of cellular processes, as **Eu1d** must be able to enter the cells in order to undergo uncaging by intracellular β-galactosidase (Figure S14). **Eu1d** uptake could potentially take place via a galactose-dependent transport mechanism[23] or passive diffusion through the cell membrane.[3c] The bulk of **Eu2** formed from **Eu1d** was localized in the extracellular milieu. As β-galactosidase was intracellular throughout the experiment (Figure S14), the participation of one or more of the bacterial efflux systems[24] in the clearance of **Eu2** is possible.

Finally, we wanted to establish whether our probes could simultaneously detect two analytes in parallel. To address this, we incubated the H_2O_2 probe **Tb1a** with 50 or 200 μM H_2O_2 in the presence of the fluoride probe **Eu1c** and 200 μM TBAF. With increasing $[H_2O_2]$, a concentration-dependent increase in the Tb emission bands (545 and 490 nm) was observed. This increase was similar to that for the sample lacking TBAF. Control experiments with TBAF but without H_2O_2 gave only background signal levels. As the 592 and 614 nm Eu emissions overlap with the Tb $^5D_4 \rightarrow {}^7F_J$ bands (J = 4, 586 nm; J = 3, 621 nm), we monitored the $^5D_0 \rightarrow {}^7F_3$ (655 nm) Eu band, to which the Tb contribution is negligible.[1,2] The intensity of this emission band was $[H_2O_2]$-independent and similar to that observed in a control experiment with **Eu1c** and $[F^-]$ = 200 μM (Figure 5).

In conclusion, a new design strategy for responsive Ln-based luminescent probes that opens the door to probes with very low background, ease of synthesis, versatility, and high analyte specificity has been developed. Current work is focused on the development of probes for additional analytes and optimization of Ln sensitization by shortening the Ln−antenna distance and thus increasing the quantum yield.[25] We are also exploring alternative antenna-forming reactions.[26] We have demonstrated the versatility of our design by developing probes for the detection of chemically quite distinct species as well as enzymatic activity. In view of the abundance of chemoselective reactions mediated by low-molecular-weight species (e.g., Hg^{2+}, H_2S, $O_2^{\bullet-}$)[27a−c] and enzymes (e.g., hydrolases, oxidoreductases)[27d−g] that reveal a phenolic OH group, we are confident that this design will prove to be exceptionally broad and useful in many new areas as well as those where Ln emission regulation is required.

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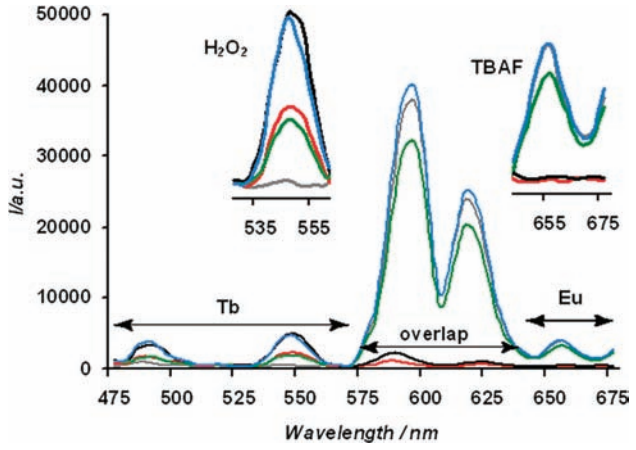

Figure 5. Simultaneous detection of two analytes. The 490 and 545 nm Tb emission bands report on $[H_2O_2]$, and the 655 nm Eu band reports on $[F^-]$. Gray, 200 μM F^-; red, 50 μM H_2O_2; black, 200 μM H_2O_2; green, 200 μM F^- + 50 μM H_2O_2; blue, 200 μM F^- + 200 μM H_2O_2 (10 μM **Tb1a**, 10 μM **Eu1c**, pH 7 HEPES buffer, 1 h, r.t., λ_{ex} = 356 nm, 50 μs delay, 1050 μs sample window).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures, characterization of all new compounds, Figures S1−14 and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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