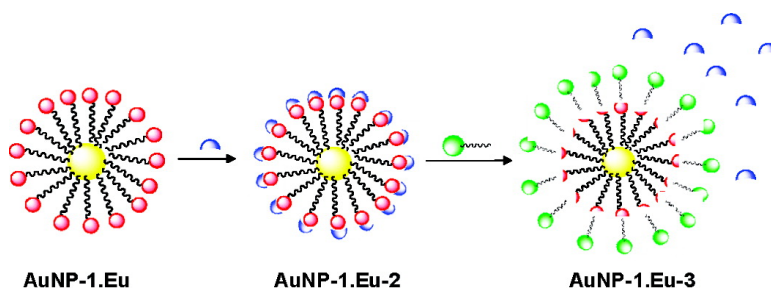


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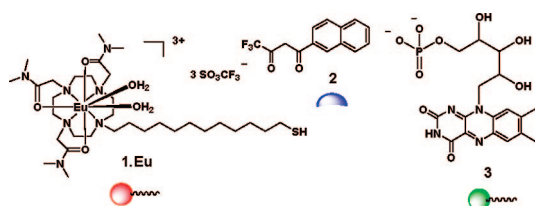
Lanthanide Luminescent Displacement Assays: The Sensing of Phosphate Anions Using Eu(III)–Cyclen-Conjugated Gold Nanoparticles in Aqueous Solution

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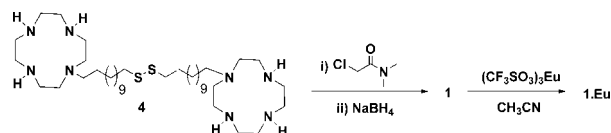
Designing functional hybrid nanomaterials and supramolecular systems is of great current interest,¹ particularly for molecular recognition and sensing,² as drug delivery systems,³ and for catalysis.⁴ Gold nanoparticles (AuNPs), have attracted much attention in the development of such devices, because of their biocompatibility, unique size- and shape-dependence, and optoelectronic properties.⁵ Concomitantly, lanthanide ion complexes have been studied as tools for optical imaging and sensing of biological species.⁶ The lanthanides have many unique photophysical properties, such as long-lived excited states and sharp line-like emission bands that occur at long wavelengths.^{7,8} These are particularly attractive features as they overcome autofluorescence and light scattering often associated with commonly used fluorophores employed in chemical biology. However, to the best of our knowledge, the combination of these unique luminescent properties with the use of AuNPs for luminescent sensing of biological substrates has not been achieved to date.⁹



Herein, we present **1.Eu**, a heptadentate macrocyclic Eu(III) cyclen conjugate possessing an alkyl thiol group, which enables the adsorption of **1.Eu** onto the surface of gold nanoparticles for the formation of water soluble gold nanoparticles **AuNP-1.Eu**. The formation of ternary complexes, or a self-assembly, between **AuNP-1.Eu** and the β -diketone antenna **2**, at pH 7.4 (0.1 M HEPES) gives rise to the formation of **AuNP-1.Eu-2**, which is highly luminescent upon excitation of the antennae. We demonstrate that these luminescent **AuNPs** can be used in the sensing of biologically relevant phosphates such as flavin monophosphate, **3**, through the displacement of **2** by **3**.

The synthesis of **1** (see Supporting Information) involved the initial formation of **4** in 70% yield, Scheme 1.¹⁰ The alkylation of the remaining six amines of **4** was achieved in CHCl_3 using 2-chloro-*N,N*-dimethylacetamide, followed by reduction of the disulfide moiety using excess NaBH_4 . This gave **1** in 52% yield after precipitation from Et_2O . The corresponding Eu(III) complex **1.Eu** was formed by refluxing **1** with 1.1 equiv of $\text{Eu}(\text{CF}_3\text{SO}_3)_3$ overnight in CH_3CN (see Supporting Information). ^1H NMR analysis confirmed the formation of paramagnetic complex **1.Eu**, and elemental analysis showed the presence of two metal-bound water molecules. This (the hydration state, q) was further confirmed by measuring the excited-state lifetimes of **1.Eu** in H_2O and D_2O , which gave $\tau_{\text{H}_2\text{O}} = 0.39$ ms and $\tau_{\text{D}_2\text{O}} = 0.89$ ms and $q \approx 1.5$, upon excitation at 396 nm.^{8d} The synthesis of **AuNP-1.Eu** was achieved

Scheme 1. Synthesis of **1** and the Eu(III) complex **1.Eu**



using the two-phase Brust method followed by the methods of Caruso et al. and Lennox et al., which gave DMAP-stabilized gold nanoparticles (**DMAP-AuNP**).¹¹ Exchange of DMAP on the surface of **AuNPs** with **1.Eu** was easily achieved by mixing **1.Eu** and **DMAP-AuNP** with stirring the resulting solution for 12 h at room temperature. Any unbound **1.Eu** was then removed using Sephadex G15 size exclusion chromatography. The resulting water-soluble gold nanoparticles (shown as cartoon in Figure 1) were found to be stable for many months when kept at 4 °C in aqueous-buffered solution at pH 7.4.

AuNP-1.Eu was characterized using UV–visible spectroscopy, where the surface plasmon resonance band, found at 520 nm for **DMAP-AuNP**, was slightly blue-shifted to 510 nm, Figure S1. **AuNP-1.Eu** was also characterized by TEM, which showed spherical nanoparticles with an average diameter of 5 nm and no evidence of aggregation, Figure 2A. Furthermore, the interparticle spacing agreed well with that predicted for capping ligands of such a chain length.

The excited states of lanthanides such as Eu(III), Tb(III), and Yb(III) are known to be quenched by O–H vibrational deactivation from water,⁶ which is particularly efficient in coordinatively unsaturated systems.¹² However, the displacement of such waters by appropriate antennae, leads to the formation of luminescent ternary complexes upon sensitization of the Eu(III) excited-state by the antennae.^{7,8} This was found to be the case for **1.Eu-2**, which gave $\tau_{\text{H}_2\text{O}} = 0.28$ ms¹ and $\tau_{\text{D}_2\text{O}} = 0.30$ ms¹, and $q \approx 0$, which is similar to that previously reported for related system.^{12a} In the case of **AuNP-1.Eu** (0.2 μM) no significant Eu(III) emission was observed in water at pH = 7.4 (0.1 M HEPES) (Figure 2B) upon direct excitation at 395 nm. However, the luminescence decay

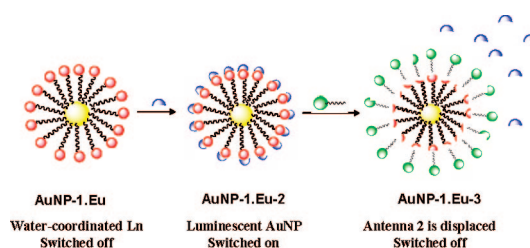


Figure 1. The self-assembly formation between the nonluminescent **AuNP-1.Eu** and **2**, to give **AuNP-1.Eu-2**. The sensing of flavin **3** occurs by the displacement of **2** and the formation **AuNP-1.Eu-3**.

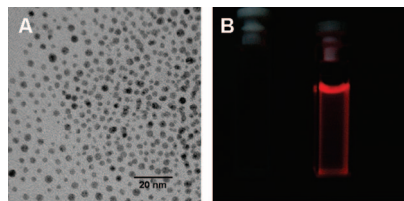


Figure 2. (A) TEM image of AuNP-1.Eu. (B) Emission of AuNP-1.Eu (switched off, left), before the addition of the antenna **2** and after (switched on, right) the formation of the AuNP-1.Eu-2 self-assembly.

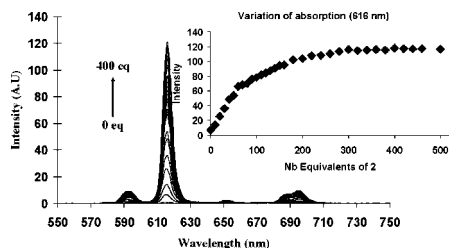


Figure 3. The changes in the Eu(III) emission of AuNP-1.Eu (0.2 μ M) upon titration in H₂O at pH = 7.4 (0.1 M HEPES) with **2**. The inset shows the changes in the 616 nm transition vs equivalents of **2**.

observed was best fitted by a double exponential, with $\tau_1 = 0.19$ ms and $\tau_2 = 0.67$ ms, respectively.

The formation of the desired ternary complex between AuNP-1.Eu and **2**, was indicated by the appearance of red Eu(III) emission that was readily visible to the naked eye under a UV-lamp, Figure 2B (see Figure S2 for **1.Eu** and **2**). The Eu(III) emission from these gold nanoparticles was also clearly visualized in the solid state using confocal fluorescence microscopy (Figure S3). Again, luminescence decay that were best fitted to a double exponential were obtained, giving $\tau_1 = 0.12$ ms and $\tau_2 = 0.47$ ms, respectively, when exciting at the antenna at 336 nm or at the metal center at 395 nm which suggest some quenching of the Eu(III)* by AuNP.

The “switching on” of the Eu(III) emission, upon formation of AuNP-1.Eu-2 from AuNP-1.Eu (0.2 μ M) was also monitored by luminescence titrations using **2** with $\lambda_{\text{ex}} = 336$ nm. Here the Eu(III) emission of AuNP-1.Eu-2 was evident from the appearance of line-like emission bands at 595, 616, 685, and 700 nm, respectively, assigned to the deactivation of the Eu(III) excited state $^5D_0 \rightarrow ^7F_J$ ($J = 1, 2, 3, \text{ and } 4$), Figure 3. Of these, the hypersensitive $\Delta J = 2$ transition, centered at 616 nm, gave rise to the greatest changes in the Eu(III) emission. This is indicative of the direct coordination to Eu(III) and the displacement of the two metal-bound water molecules from each center.⁶ The binding isotherm for the formation of AuNP-1.Eu-2 is shown as an insert in Figure 3. As the binding is in a 1:1 stoichiometry between each gold conjugated **1.Eu** and **2**, we can estimate the number of bound Eu(III) complexes per AuNP to be ca. 230. The AuNP-1.Eu-2 self-assembly is now primed to sense suitable coordinating ligands such as biologically relevant anions capable of displacing **2**. With this in mind, a selection of anions such as phosphates, carboxylates, etc. were investigated by carrying out luminescent titrations on AuNP-1.Eu-2 at pH 7.4 (Figures S4–S16). The results from these displacement assays¹³ are summarized in Figure S4, and demonstrated that phosphate containing molecules such as AMP, ADP, ATP, cyclic AMP, or NADP, quenched the Eu(III) luminescence, where $\Delta J = 2$ was reduced in intensity between 20–55% after adding 500 equiv of these anions. Carboxylate containing creatine, coumaric, and panthoic acid gave rise to minor luminescent changes while Na₂CO₃ and H₂PO₄ resulted in only 25% quenching. In contrast to these results, flavin mononucleotide **3** (Figure S5), which plays a

key role in many electron transfer, oxidation, and dehydrogenation processes, was the only anion investigated that could effectively displace **2** from AuNP-1.Eu-2. This resulted in almost complete quenching of the Eu(III) emission and the formation of AuNP-1.Eu-3 (Figure 1 and Figure S5), which shows the selective sensing of **3** by AuNP-1.Eu-2, but **3** is unable to sensitize the Eu(III) excited state. With the aim of exploring the sensing potential of AuNP-1.Eu-2 further, we also carried out titrations using calf thymus (*ct*) DNA in buffered pH 7.4 solutions. The results showed that AuNP-1.Eu-2 bound to DNA where the Eu(III) emission was only ca. 30% quenched (0 \rightarrow 20 equiv of *ct*-DNA). These results suggest interaction with DNA, most likely through the phosphate backbone, resulting in partial displacement of **2**. The selective displacement of **2** from AuNP-1.Eu-2 is currently the under investigation in our laboratory. In summary, we have developed the first example of structurally defined luminescent lanthanide AuNP and demonstrated the sensing of anions through the displacement of sensitizing antennae at the surface of the AuNP.

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Supporting Information Available: Synthesis and characterization of **1** and **1.Eu**; Figures S1–S10. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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