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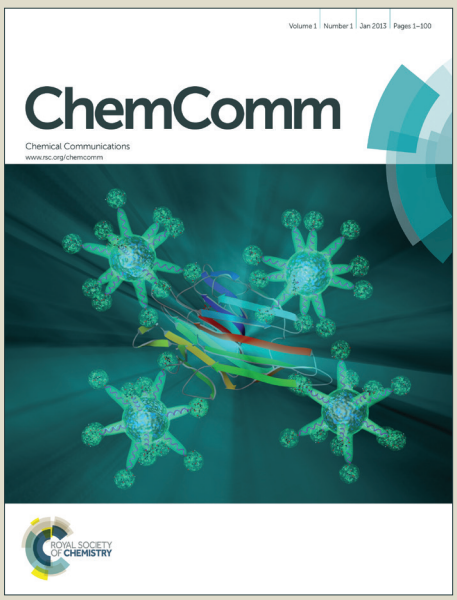

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ARTICLE TYPE

A lanthanide based sensor for the time-gated detection of hydrogen sulfide

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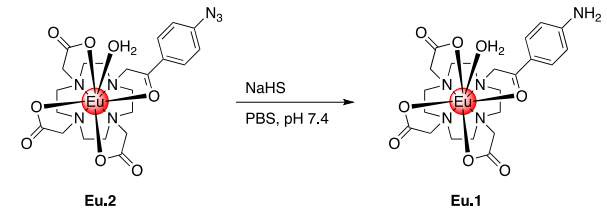
A kinetically stable Eu(III) complex for the detection of sulfide in water is reported. The probe shows excellent selectivity and fast reaction time. Its long-lived luminescence makes this the first probe for the time-gated detection of sulfide in complex biological samples.

The role of hydrogen sulfide in living organisms has received increasing attention in the recent years due to the importance of this molecule as a potential endogenous gasotransmitter.[1, 2] H_2S is produced by vascular smooth muscle tissue and has been shown to act as a vascular relaxant, and to be implicated in cardiovascular regulation and the inflammation response.[3] Recently it has been demonstrated that hydrogen sulfide is produced in mitochondria of mammalian cells under hypoxic conditions and that its presence triggers an increase in ATP production, thus acting as an energy regulator under stress conditions.[4, 5]

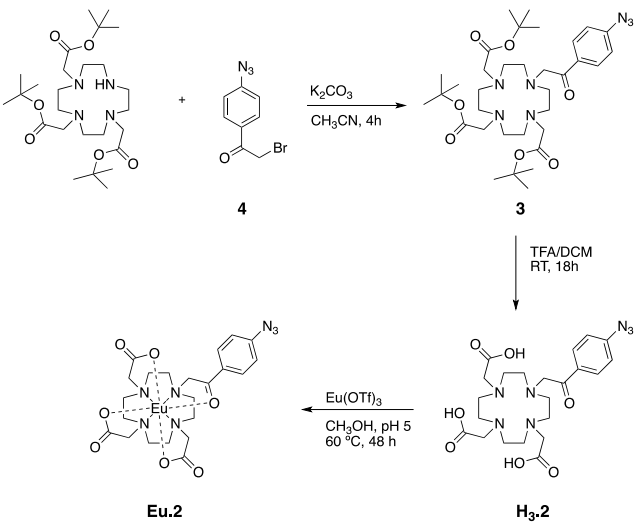
A number of fluorescent molecules for the non-invasive detection of H_2S have been reported to date; these exploit copper sulfide precipitation, sulfide mediated reduction of nitro and azide groups and nucleophilic addition of S^{2-}.[6-15] Despite the increasing effort in the preparation and study of suitable fluorescent H_2S sensors, there is still considerable debate and doubt about the effective concentration in living organisms.[16, 17] One major difficulty when working with biological samples is that inner filter and auto-fluorescence effects can introduce considerable complications, limiting the prospect of a quantitative measurement. A sulfide sensitive fluorescence detector with a long luminescence lifetime would be enormously beneficial to the field. This would allow background-free luminescence imaging even in highly complex biological samples obtaining luminescence intensity measurements with high signal-to-noise ratio and hence improved sensitivity. Lanthanide containing probes have been extensively used as sensors for halide and oxyanions in aqueous media showing selectivity based on the metal, on the ligand periphery and on guest chirality.[18-20] Furthermore, kinetically stable lanthanide complexes have found widespread applications in fluorescence assays and as building blocks for the preparation of responsive supramolecular architectures and heteromultimetallic complexes.[21-26] More recently, reaction based lanthanide probes able to detect glutathione and hydrogen peroxide have been reported.[27, 28]

In this paper we describe the synthesis and the sensing properties of a fluorescence sensor based on a Eu(III) chelate. The sensor emits in the red region and has a long luminescence



Scheme 1. Transformation of the antenna group by azide to amino group reduction using NaHS in PBS buffer.

lifetime (of millisecond order) facilitating the use of time-gating techniques to separate the desired signal from autofluorescence and scattered light. Since *f-f* transitions are formally forbidden, it has become commonplace to use strongly absorbing chromophores to sensitise lanthanide emission. With europium, such sensitisation commonly occurs through the triplet state, and use of chromophores with small triplet-singlet energy gaps can facilitate excitation with visible light.[25, 29-31] This combination of long wavelength excitation and long-lived red luminescence from europium (III) potentially allows us to obtain high signal-to-noise ratios even in tissue samples. We reasoned that an aryl ketone derived sensitising chromophore with a pendent azide group would allow H_2S to modulate the luminescence intensity from a lanthanide ion.



Scheme 2.

In such a system, the sensing mechanism relies on the H_2S reduction of an azide group, forming Eu.**1** from Eu.**2**, with a consequent change in the energy transfer efficiency between the antenna chromophore and the emitting lanthanide centre (see Scheme 1).

Scheme 2 shows the synthetic strategy used to access Eu.**2**. Compound **3** was obtained by reaction of 4-azidophenacylbromide[32] with the tris tert-butyl ester of DO3A (**5**).[30] Treatment of **3** with trifluoroacetic acid catalysed cleavage of the tert-butyl groups and yielded H_3.**2**, whose subsequent complexation with Eu(III) afforded Eu.**2**. The DO3A macrocycle with its three carboxylate arms ensures thermodynamic and kinetic stability, while the azido-acetophenone chromophore offers long wavelength sensitisation whose efficiency is dependent on the donor ability of the aryl group.

Beeby et al. have previously shown that aryl ketones are effective sensitisers of lanthanide luminescence, and demonstrated that the small singlet-triplet energy gap in such systems offers the potential for excitation of such chromophores at relatively long wavelengths.[33, 34] Furthermore, they also showed that the electron donating ability of substituents on such chromophores could be correlated with the efficiency of sensitisation of the lanthanide emission. The photophysical properties of Eu.**2** were therefore studied in detail, revealing that the azidophenacyl chromophore is an effective sensitiser of lanthanide luminescence (Fig 1a). Time-resolved measurements and proton NMR were used to probe the solution state structure of Eu.**2**. Key data are shown in Table 1, with values for Eu.**1** shown for comparison. Measurements of the luminescence lifetime in H_2O and D_2O were used to calculate q, the number of inner sphere solvent molecules close to the lanthanide centre.[35] For Eu.**2**, q = 1.1, implying that the phenacyl group can coordinate to the lanthanide. Paramagnetic ^{1}H-NMR revealed that the square antiprismatic (SAP) isomer dominates in solution although the twisted square antiprismatic (TSAP) isomer is also present (see ESI). These isomers are in slow exchange on the NMR timescale, implying that coordination of the carbonyl to the metal centre creates a barrier to rotation. Eu.**2** shows a quantum yield of 0.45 % and light excitation at 350 nm (ε = 6300 M^{-1} cm^{-1}). This is ascribed to a charge transfer state centred on the chromophore as evidenced by the solvatochromic behaviour displayed by the UV-Vis absorption of the complex (see ESI).

Based on these observations we envisaged that transformation of the azide group into the electron donating amino group would be reflected in a change of the energy transfer efficiency to the lanthanide centre. Indeed, upon addition of NaHS to Eu.**2** in 10 mM PBS buffer at pH 7.4, we observed an up to 20 fold increase in the characteristic europium fluorescence. Furthermore the product of this *in situ* reduction Eu.**1** shows a red-shifted UV-Vis absorption profile as expected from a chromophore with a higher degree of charge transfer character (see ESI).[33]

In order to demonstrate that the result of the enhanced fluorescence signal is indeed due to the formation of Eu.**1**, an HPLC profile of a solution of Eu.**2** upon addition of NaHS was recorded after a few hours. This shows that there is only one reaction product forming and that this is indeed the reduced complex **Eu.1** as unequivocally demonstrated also by ES-MS of the eluted HPLC peak (see ESI).

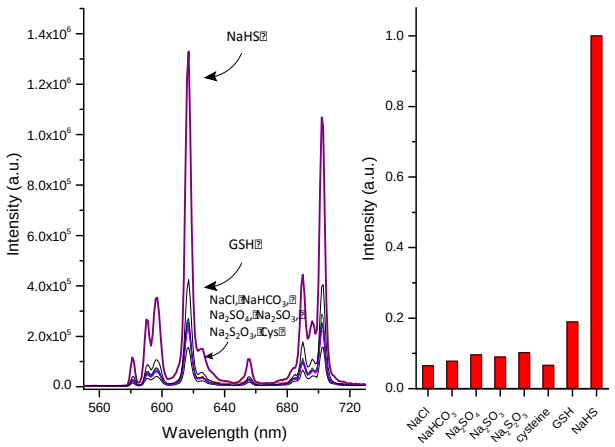


Figure 1. Change in overall luminescence of **Eu.2** (10 uM) showing corrected emission spectra upon addition of NaCl (100 mM), $NaHCO_3$ (25 mM), Na_2SO_4, Na_2SO_3, $Na_2S_2O_3$, Cysteine, GSH (1 mM) and NaHS (250 uM) in 10 mM PBS pH 7.4, 295 K.

A study of the selectivity of **Eu.2** towards H_2S was then performed; the fluorescence response was tested in the presence of various biologically relevant anions and putative reducing species (see Figure 1). No fluorescence response was comparable with the one obtained using 250 uM of NaHS vs. a probe concentration of 10 uM. Almost no response was observed using Cl^- (100 mM), HCO_3^- (25 mM) or using other possible reducing agents (1 mM). Of the species tested, only glutathione (GSH) triggered a fluorescence signal enhancement that nonetheless was only two-fold using four times the concentration of NaHS, thus not affecting the selectivity of the probe (see Figure 1b).

The response time is an important parameter when assessing a luminescent sensor, particularly in this case given the transient nature of sulfide in living organisms.[16] In order for such a probe to have a potential use in a biological environment, fast reaction times are required. The reduction of **Eu.2** by NaHS is complete in ca. 30 minutes with a six-fold enhancement just after 5 minutes (see ESI). This makes this probe potentially suitable for real-time monitoring of sulfide levels in biological specimens. In particular, varying the concentrations of NaHS resulted in a linear response of the lanthanide centred luminescence over a wide concentration range (between 80nM and 1μM), as can be seen from the data in Figure 2. Given the free ninth axial coordination site on the metal centre, direct interaction of the anion with Eu(III) must be considered. Such effect has widely been exploited for anion binding using lanthanide complexes based on similar architectures.[18] However, in this case, the relative intensities of the Eu(III) emission bands remained unchanged on addition of NaHS while the change in luminescence lifetime was very small,

Table 1.Excited state lifetimes, number of inner sphere solvent molecules (q) and quantum yield for **Eu.2** recorded in H_2O (λ_{ex} = 350 nm, 295 K).

Complex	τ (H_2O)/ms	τ (D_2O)/ms	*q*	Φ(H_2O)/%
Eu.2	0.60	2.05	1.1	0.45
Eu.1	0.44	0.92	1.1	0.56

thus indicating that the metal environment remains essentially unchanged throughout the titration hence ruling out sulfide coordination and solvent displacement.

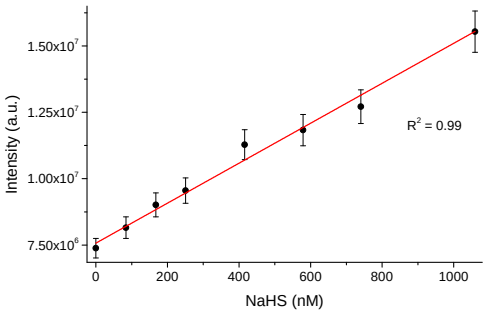


Figure 2. Luminescence response of **Eu.2** upon addition of NaHS in 10 mM PBS buffer pH 7.4, 295 K, λ_{ex} = 350 nm.

Given the observed long lifetimes we tested the ability of Eu.**2** to probe NaHS in serum, as a proof of concept application for the determination of sulfide in complex biological samples. While the signal originating from Eu.**2** is subsumed by the much stronger signal of fluorescent species present in serum, a pure Eu.**2** spectrum can be observed in a time-gated luminescence spectrum. Furthermore, addition of NaHS causes a linear increase in the luminescence signal thus providing an effective way to directly determine sulfide in biological samples.

Conclusions

A water soluble, kinetically stable Eu(III) based luminescent probe has been synthesised and its luminescence change upon addition of sulfide assessed. The probe selectively responds to sulfide in the nanomolar range, its luminescence signal is long-lived and hence suitable for time-gated detection. Furthermore the fast response times and long wavelength excitation render this a suitable candidate for imaging of live biological samples where photodamage and sample autofluorescence can constitute an issue. We are currently working on developing and applying the probe for imaging microscopy.

Notes and references

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