Cite this: *Chem. Commun.*, 2011, **47**, 6810–6812

www.rsc.org/chemcomm

COMMUNICATION

Sensing of biologically relevant d-metal ions using a Eu(III)-cyclen based luminescent displacement assay in aqueous pH 7.4 buffered solution†

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Received 30th March 2011, Accepted 28th April 2011

DOI: 10.1039/c1cc11810b

1-Eu-BPS was developed as a luminescent lanthanide sensor for use in displacement assays for detection of d-metal ions by monitoring the changes in the europium emission, which was quenched for iron(II), with a detection limit of ~ 10 pM ($0.002 \mu\text{g L}^{-1}$) for Fe(II) in buffered pH 7.4 solution.

Over the past decades the importance of metals in biology has been increasingly recognised.¹ Approximately one third of the human proteome contains metal cations, either in the form of cofactors, with catalytic function, or as structural support elements. Moreover, a growing number of diseases have been identified that are characterised by metal imbalance in cells and tissues. Hence, the identification and quantification of these in the physiological environment is of great current importance.² Recently, the use of lanthanide luminescent systems as sensors for metal ions has been explored by us and others.³ Due to their long wavelength emission and long excited state lifetimes these are ideal candidates for use in biological environments.⁴ Recently we developed the heptadentate cyclen Eu(III) complex **1-Eu**, Scheme 1, possessing an alkyl thiol group, which facilitates its adsorption onto gold nanoparticles, as well as onto flat

gold surfaces, at the same time as removing an effective N–H oscillator from the cyclen structure.⁵ Herein we demonstrate the use of this structure for application in lanthanide luminescent sensing for metal cations using displacement assays. The formation of a ternary complex between **1-Eu** and the water soluble antenna 4,7-diphenyl-1,10-phenanthroline-disulfonate (**BPS**) gives rise to the formation of **1-Eu-BPS**, a red emitting Eu(III)-luminescent species, Scheme 1, upon excitation of the **BPS** antenna. **BPS** has previously been shown to effectively sensitise Eu(III),⁶ possessing good water solubility, at the same time as being a known ligand for the selective colorimetric sensing of Fe(II).⁷ We foresaw that the Eu(III) emission of **1-Eu-BPS** could be modulated, or “switched off” through the displacement of the **BPS** antenna^{4e–g,8} in the presence of Fe(II); for which it has high affinity. The Fe(II) induced displacement would be reflected in the emission of the hypersensitive $^5\text{D}_0 \rightarrow ^7\text{F}_2$ transition of Eu(III) in particular, upon formation of **Fe-BPS**₃ and **1-Eu**, the latter being poorly luminescent in the absence of the antenna, possibly enabling us also to achieve enhancement in the detection limit of **BPS** for Fe(II), over that currently seen for colorimetric sensing of Fe(II).⁷

The synthesis and characterisation of **1** and its Eu(III) complex, **1-Eu**, have been previously described by us.^{5a} By measuring the excited state lifetimes of **1-Eu** in H₂O and D₂O, the hydration state (*q*), or the number of metal bound water molecules was confirmed as two in aqueous solution.^{5a} The formation of a ternary complex between **BPS** and **1-Eu** to give **1-Eu-BPS**, Scheme 1, was next monitored in pH 7.4 buffered solution by observing the evolution of the sensitised Eu(III) emission at long wavelengths. The results (see Fig. S1–S4, ESI†) clearly demonstrated that **BPS** functions as an efficient sensitizer for Eu(III), occurring through an energy transfer process from S₁ via the T₁ of the antenna to the Eu(III) $^5\text{D}_0$ excited state, with concomitant deactivation to the $^7\text{F}_J$ ground states (*J* = 0–4), upon excitation at **BPS** λ_{max} at 278 nm. As expected, no significant emission occurred from **1-Eu** in the absence of this antenna, but upon formation of **1-Eu-BPS**, a *ca.* 2.5-fold emission enhancement was observed for $\Delta J = 2$ (Fig. S4, ESI†), along with a minor change in the $^7\text{F}_2/^7\text{F}_1$ emission ratio from 6.0 ± 0.2 to 6.5 ± 0.1 . This indicates direct binding of **BPS** to the Eu(III). Concomitantly, the fluorescence emission was also monitored (Fig. S3, ESI†). The observed changes in the **BPS** fluorescence and the Eu(III) emission (*e.g.* Fig. S3 and S4, ESI†) were analysed, and both displayed

Scheme 1 Schematic representation of the displacement assay developed for the selective sensing of Fe(II) using **1-Eu-BPS** in aqueous solution.

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† Electronic Supplementary Information (ESI) available: Experimental part, detailed photophysical studies. See DOI: 10.1039/c1cc11810b

Fig. 1 The changes in the absorption spectrum of **1-Eu-BPS** (10^{-5} M) in Hepes-buffered solution (pH 7.4) upon addition of Fe(II) (0 $\rightarrow$ 4 equiv.), clearly demonstrating the formation of the MLCT band.

a maximum at ~ 1 equivalent of **BPS**, revealing the formation of the 1:1 ternary complex **1-Eu-BPS** (Scheme 1). Further evidence for the formation of **1-Eu-BPS**, and thus the direct binding of the antenna to the Eu(III) centre, was also obtained by determining q values in the presence of **BPS**, which gave lifetimes of 0.49 ± 0.01 ms and 0.60 ± 0.01 ms in H_2O and D_2O , respectively ($\lambda_{\text{ex}} = 278$ nm), from which a $q \approx 0$ was determined. The binding constant for the formation of **1-Eu-BPS** was determined by fitting the changes in the Eu(III) emission using both linear and non-linear regression analysis, from which binding constants, $\log K$, of 8.2 ± 0.9 and 7.5 ± 0.8 were determined, respectively (Fig. S5, ESI†).

The photophysical properties of **1-Eu-BPS** in the presence of various metal cations ($\text{M(II)} = \text{Ca, Cd, Co, Cu, Fe, Mg, Ni}$ and Zn ; $\text{M(III)} = \text{Cr, Fe}$) were next evaluated, by observing the changes in absorption, excitation and in both the fluorescence and Eu(III) emission spectra of **1-Eu-BPS**. The discussion herein will focus on the spectrophotometric titrations with Fe(II), since, and as discussed above, **BPS** is a selective colorimetric ligand for Fe(II).⁷ The changes observed in the **1-Eu-BPS** absorption spectra are shown in Fig. 1. The absorption spectrum of the ternary complex consisted of a broad band with $\lambda_{\text{max}} = 275$ nm, attributed to the conjugated structure of **BPS** i.e., intra-ligand $\pi-\pi^*$ absorption.⁹ Upon addition of Fe(II), this λ_{max} was red shifted by ca. 5 nm and a new metal-to-ligand charge transfer (MLCT) absorption band ($d-\pi^*$)^{7,9a} was observed with a maximum at 535 nm. These changes can be viewed as a successful displacement of the **BPS** antenna, which was accommodated with a visible colorimetric change (from a colourless to an orange-red solution), indicative of the formation of the **Fe-BPS₃**. These changes were analysed by fitting the global changes in Fig. 1 using non-linear regression analysis (Fig. S6, ESI†). The speciation-distribution diagram resulting from these fits showed the presence of two major species in solution (Fig. S7, ESI†), assigned to **1-Eu-BPS** and **Fe-BPS₃**, respectively. Of these, the former was the most predominant species upon addition of 0.2 equivalents of Fe(II), while at 0.33 equivalents of Fe(II), **Fe-BPS₃** was formed in 98% yield. From this analysis we determined the binding constants (expressed as $\log \beta_{\text{M:L}}$) for the formation of the Fe(II) complex as $\log \beta_{1:3} = 20.0 \pm 0.6$, which is in good agreement with that reported by Bell *et al.*^{9b}

Fig. 2 The changes in the Eu(III) emission of **1-Eu-BPS** in Hepes-buffered solution (pH 7.4) upon addition of Fe(II) (0 $\rightarrow$ 4 equiv.). Inset: the changes in the Eu(III) $^5\text{D}_0 \rightarrow ^7\text{F}_2$ transition vs. equiv. of Fe(II) added.

In parallel to the changes observed in the absorption spectra, the fluorescence (Fig. S8, ESI†) and the Eu(III) emission of **1-Eu-BPS**, Fig. 2, were also monitored. Here, the emission intensity in both cases decreased significantly (with the ratio $^7\text{F}_2/^7\text{F}_1$ going back to 6.0) before reaching a plateau at ~ 0.33 equivalents of Fe(II) (see the inset in Fig. 2 and Fig. S8, ESI†). The quenching observed upon addition of Fe(II) in both the fluorescence and Eu(III) emission confirmed again the displacement of the antenna from the ternary complex, and thus the formation of **Fe-BPS₃** in solution. This is to be expected, as the displacement of the antenna from the Eu(III) centre is accompanied by quenching in both the antenna (Fig. S8, ESI†) and Eu(III) emission.

Changes in the Eu(III) sensitisation process throughout the Fe(II) titration were also monitored by recording the excitation spectrum at 617 nm ($^5\text{D}_0 \rightarrow ^7\text{F}_2$ transition). In the absence of Fe(II) the spectrum closely matched the absorption spectrum of the antenna with $\lambda_{\text{max}} = 282$ nm, while the band at 247 nm was characteristic of **1-Eu** (Fig. 1; Fig. S9a, ESI†). Interestingly, the ratio between the intensities at 282 nm and 247 nm decreased from ca. 1.3 to 0.6 upon addition of Fe(II) (Fig. S9b, ESI†). The latter ratio being identical to that calculated using the excitation spectrum of **1-Eu** in the absence of antenna. This measurement can thus be used as an additional tool for monitoring the displacement of **BPS** from **1-Eu-BPS** by Fe(II). From this displacement assay, we were able to use the changes in the Eu(III) emission to determine the binding constant for the formation of the **Fe-BPS₃** complex, by fitting the global changes using non-linear regression analysis. This gave $\log \beta_{1:3} = 19.0 \pm 1.5$, while linear-regression analysis gave $\log \beta_{1:3} = 20.0 \pm 0.2$, which correlates well with that found in the ground state changes. In a similar manner, we were able to fit the changes in the fluorescence emission spectra, from which a $\log \beta_{1:3} = 22.0 \pm 2.5$ was determined. Furthermore, it demonstrates that **1-Eu-BPS** has good sensitivity for Fe(II), and importantly, that Fe(II) can be sensed by employing different wavelengths and excited state time-channels using **1-Eu-BPS**. Moreover, using this displacement assay, we were able to achieve the lowest limit of detection (LOD) of 10 pM for Fe(II) ($0.002 \mu\text{g L}^{-1}$, using 2.7 mL samples) by monitoring the changes in the Eu(III) emission (Fig. S10–S13, ESI†). In contrast, using ion-exchanger phase absorptivity, a LOD value of $0.01 \mu\text{g L}^{-1}$ has been reported using 80 mL samples.^{7a}

Fig. 3 Bar chart diagrams showing the response to: (A) absorbance (at 535 nm), (B) fluorescence and (C) Eu(III) emission, of **1-Eu-BPS** in the presence of 0.33 equiv. of selected d-metal and group II metal ions, at pH 7.4.

This clearly demonstrates the sensitivity of **1-Eu-BPS**, and the advantage of its use in luminescent based displacement assays. The sensing of Fe(II) was also investigated in the presence of various anions: 30 mM carbonate, 2.3 mM lactate, 0.13 mM citrate and 0.90 mM phosphate. Here, no significant changes were seen in the absorption and the fluorescence emission spectra of **1-Eu-BPS** to that above; while the Eu(III) emission was slightly affected, being ca. 5–10% reduced in intensity (see ESI†, Fig. S14–S16).

To establish the selectivity for the sensing of Fe(II) we also investigated the response of **1-Eu-BPS** towards the other metals mentioned above (see Fig. S17–S19, ESI†). The overall (relative) changes observed in the absorption, fluorescence and the Eu(III) emission are presented in Fig. 3 as bar charts. Due to the similar affinity of ions such as Co(II), Ni(II) and Cu(II) for polypyridyl ligands, we anticipated that these ions would give rise to some modulations in the spectroscopic properties of **1-Eu-BPS**. The results from these titrations demonstrate that in comparison to Fe(II), only minor changes were observed in the absorption spectra of **1-Eu-BPS**, Fig. 3A. However, a similar quenching effect was seen for these ions as previously seen for Fe(II) in both the fluorescence and the Eu(III) emission spectra (Fig. 3B and C, respectively), which prevented competitive studies from being carried out. In comparison, Zn(II) led only to a partial quenching in the Eu(III) emission, which was accompanied with enhancement in the fluorescence of the **BPS** (Fig. 3B and C). By observing the absorbance and fluorescence excitation spectrum at 617 nm, we confirmed the displacement of the antenna from **1-Eu-BPS** (Fig. S20, ESI†). However, the affinity of Zn(II) for **BPS** is lower than that for Fe(II)^{9b} and thus addition of Fe(II) to this solution leads to the formation of **Fe-BPS₃** with identical changes to that seen in Fig. 1 and 2, demonstrating the selectivity of **1-Eu-BPS** for Fe(II) over Zn(II). Some changes were also observed in the Eu(III) emission upon titration with Ca(II). However, these were attributed to a possible interaction of Ca(II) with the sulfonate groups of **BPS**, and not due to displacement of the antenna (Fig. S21, ESI†).

In summary, we have developed, to the best of our knowledge, the first example of lanthanide luminescent displacement assays for biologically active d-metal cations. We have shown that **1-Eu-BPS** gave both good selectivity and excellent sensitivity for Fe(II) in competitive media. We are currently incorporating **1-Eu-BPS** onto gold nanoparticles⁵ and evaluating their potential use as Fe(II) displacements sensors on such a platform within biological media.

We thank the Irish Research Council for Science, Engineering & Technology (IRCSET) (OK, SC), the Swiss

National Science Foundation (SNSF) (SC) for Postdoctoral Fellowships and Science Foundation Ireland (SFI RFP 2009 and 2010 PI awards) and TCD for financial support.

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