Dalton Transactions

COMMUNICATION

A near-IR luminescent ratiometric ytterbium pH probe

Sergey Shuvaev,*[a] and David Parker*[a]

Received 00th January 20xx,
Accepted 00th January 20xx

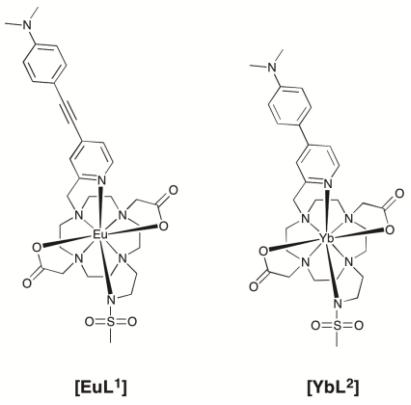
DOI: 10.1039/x0xx00000x

www.rsc.org/

The hypersensitive $^2F_{5/2}$ to $^2F_{7/2}$ transition of Yb^{3+} can be used to monitor perturbations of the coordination sphere in ytterbium(III) complexes. An envelope of Stark components gives rise to a relatively broad and asymmetric emission band, whilst changes in their relative intensity and energy enable a ratiometric response. We report a new ytterbium complex with a sulphonamide arm that binds reversibly to Yb^{3+} as a function of pH, giving rise to significant pH dependent changes in the Yb emission spectrum.

Lanthanide complexes emitting in the near-IR region have been attracting considerable attention over the last decade, owing to their potential use as bio-imaging probes[1]. It is well known that biological tissue has higher optical transmission in the near-IR region, and therefore near IR-emitting molecular probes are advantageous compared to those emitting in the visible region.[2] Among the lanthanide ions that have transitions in the near-IR region (Pr^{3+}, Nd^{3+}, Dy^{3+}, Ho^{3+}, Er^{3+}, Tm^{3+} and Yb^{3+}), ytterbium is arguably the most promising, owing to its higher emission quantum yields. Indeed, values up to several per cent in aqueous media have been reported. [3,4]

However, the immediate Yb coordination environment needs to be considered carefully, otherwise vibrational deactivation of the Yb excited state by C-H, N-H and related oscillators can limit their practical application. Thus, ligand per-fluorination has been advocated, yet impairs solubility in an aqueous medium.[5] However, quantum yields in aqueous media have been also gradually rising over the past years, even allowing live cell imaging using an ytterbium complex as a bio-imaging tag[4,6]. At the same time, there are only a few examples of responsive ytterbium probes, where emission is modulated reversibly in solution[7,8]. To the best of our knowledge, no responsive ratiometric luminescent Yb probes have been reported previously.



[EuL^1] **[YbL^2]**

Figure 1 Structures of **[EuL^1]** and **[YbL^2]**

The simplicity and hypersensitivity of the $^2F_{5/2}$ to $^2F_{7/2}$ transition makes Yb complexes attractive candidates for the development of responsive probes. Reversible changes of the metal coordination environment can induce significant changes in the emission signature of related europium complexes. [9] For example, the pH dependent reversible binding of a sulphonamide nitrogen to a central Eu ion in cyclen-based systems has been studied in depth earlier. [10,11] Sulphonamide binding was shown to lead to considerable changes in the metal-based emission spectrum. More recently, aspects of the behaviour of the europium complex **[EuL^1]** were reported. [12] However, it was found to be only very weakly emissive in the absence of added protein, and could not be used to assess pH when free in solution.

The same principle of reversible sulphonamide ligation can be employed with Yb complexes, where reversible protonation of the sulphonamide nitrogen and its substitution with a water molecule might produce sufficient changes in the ligand field,

[a] *Department of Chemistry, Durham University, South Road, Durham, DH1 3LE, UK.*
† Footnotes relating to the title and/or authors should appear here.
Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/x0xx00000x

i.e. the splitting of the $^2F_{5/2}$ to $^2F_{7/2}$ transition, to allow spectral monitoring as a function of pH.

In order to provide relatively efficient energy transfer from the antenna moiety to the metal ion, a chromophore with a lower triplet energy level has normally been employed to provide a better energy match to the lower lying $^2F_{5/2}$ state of Yb^{3+}. With these considerations in mind, **[YbL²]** was designed and synthesised.

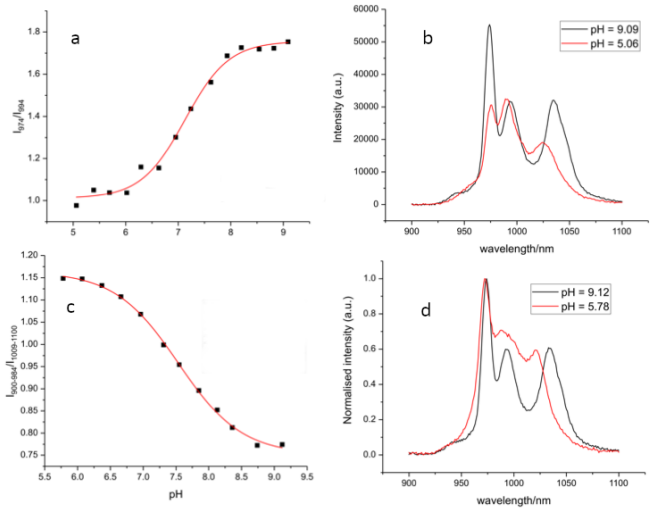


Figure 2 a,c) pH calibration curves for **[YbL²]** following the relative intensities of two bands (974 nm vs. 994 nm (a) and 900-984 nm vs. 1009-1100 nm (c)) of the $^2F_{5/2} \rightarrow {}^2F_{7/2}$ transition manifold in the emission spectrum in the presence of (a) human and (c) bovine serum albumin (I = 0.1M, 0.6 mM, λ_{ex} = 360nm, 298K). Emission spectra of **[YbL²]** at the two stated pH values in the presence of (b) human and, (d) bovine serum albumin (I = 0.1M, 0.6 mM, λ_{ex} = 360nm, 298K).

The ethyl ester of the DO2A di-ester (ESI: DO2A = 1,7-*bis*(carboxymethyl)-1,4,7,10-tetraazacyclocdodecane) was alkylated with a freshly prepared methanesulfonate derivative of the chromophore. (Scheme 1 ESI). The latter was synthesized in accordance with a Suzuki-Miyara cross-coupling reaction in a microwave reactor, using $Pd(TPP)_2Cl_2$ as a catalyst and Cs_2CO_3 as the base. The trialkylated product was purified using semi-preparative RP-HPLC. The sulphonamide moiety was introduced by reaction of N-methanesulfonyl-aziridine, prepared *in situ,* with this tri-substituted intermediate. The crude product was hydrolysed in aqueous base, neutralised and reacted with an excess of $YbCl_3$. The desired Yb complex was purified using semi-preparative RP-HPLC (Fig S1), and showed a well-defined ^1H NMR spectrum only at elevated pH values (Fig S2). At pH values below 6, considerable exchange broadening was observed.

The complex showed a typical Yb-centred emission profile, with an excitation maximum at 360 nm. The variation of pH resulted in pronounced changes in the emission pattern (Fig. 2). Indeed, quenching of the total emission intensity was observed on going from pH = 7.0 to pH = 3.0, consistent with protonation of the sulfonamide N atom followed by binding of a water molecule. Plotting the ratio between integral intensities of two bands as a function of pH gave an apparent pK_a value of 4.84(05) (Fig S3 and S4), in line with previously observed values for related bi-aryl systems [12]. At the same time, the lifetime of the excited $^2F_{5/2}$ state decreased from 3.3 µs (pH = 7.0) to 1.3 µs (pH = 2.8).

Using an empirical equation devised for analysing aqueous solutions of Yb complexes[13], $q = (k(H_2O)-k(D_2O)-0.1)$, hydration numbers of zero (pH = 7.0) and 0.4 (pH = 2.8) were determined (Table 1). The latter value is lower than that observed for the related Tb complex in the same experiment (q = 1.4) and can be explained by the smaller size of the Yb^{3+} ion, which can accommodate only one water molecule in its smaller coordination sphere. However, the lower accuracy of the aforementioned equation must be acknowledged, as the number of complexes used to derive it was smaller than for analogous expressions for Tb and Eu complexes. The non-integral *q* value may also simply reflect a slightly longer metal-water distance, as such vibrational energy quenching shows an r^{-6} dependence. [13]

Table 1 Selected photophysical parameters for **[YbL²]** (298 K, H_2O/D_2O)

	τ(D_2O)/µs	**k(D_2O)/ µs^{-1}**	**τ(H_2O)/µs**	**k(H_2O)/ µs^{-1}**	***q***
pH (pD) = 2.8	5.9	0.17	1.3	0.77	0.4
pH(pD) = 7.0	7.3	0.14	3.3	0.30	0

Binding of lanthanide complexes to proteins can perturb the position of the protonation equilibrium and significantly change the measured pK_a[14]. Such an effect should be taken into account when comparing results in salt *vs* serum, for example. In the present study, the pH-sensitive behaviour of **[YbL²]** following addition of human and bovine serum albumin was studied. In each case, an elevated pK_a value was revealed (pK_a = 7.13(05) for HSA and pK_a = 7.53(04) for addition of BSA: Fig S5-S7), whilst substantially smaller changes of the total emission intensity were observed, within the pH range examined. Examination of the excitation spectrum of **[YbL²]** in the presence of 0.6 mM HSA (Fig S10) shows a modest 8nm shift to the red of the spectral maximum at pH 8.3 (sulfonamide bound) compared to pH 5.1 (sulfonamide not bound).

At higher pH values (e.g., pH > 8) the emission signature was identical to the complex with a bound sulphonamide N atom with no added protein, whilst it was different between HSA and BSA added samples at lower pH, when the sulphonamide nitrogen atom is protonated – i.e., for **[YbL²]** without protein, with added HSA or with added BSA, (see Fig S 8-9). These changes were accompanied by variations in the relative intensities of the Yb emission bands and the frequencies of their maxima. Particularly, in the cases of added HSA and BSA, the ligand field strength was reduced upon lowering pH,

presumably due to substitution of the sulphonamide nitrogen with a carboxylate oxygen of a Glu (or Asp) side chain in the protein. Previously, we already observed different spectral signatures for the europium complex, [**Eu.L^1]** with bovine and human α_1-AGP protein with protonated sulphonamide nitrogen[12], which were attributed to differences in the amino-acid residues near the complex binding site, between bovine and human varieties. Bearing in mind that the drug-binding site I in HSA and BSA are quite different[15], the different emission patterns for **[YbL^2]** in the presence of added HSA and BSA, suggest that side chains of different amino-acids of the side chain may be bound to Yb.

In conclusion, the first ratiometric Yb pH-sensitive probe has been reported. In the presence of serum albumin proteins that shield the Yb centre from vibrational quenching by proximate water molecules, the overall emission intensity of **[YbL^2]** produces significant changes in spectral form within the working pH range. A similar strategy that explores the scope and utility of protein- bound Yb complexes could be employed in the future, in seeking to devise new ratiometric probes for cellular imaging.

We thank Durham University for a doctoral studentship (SS).

Conflicts of interest

There are no conflicts to declare.

Notes and references

1 S. Comby and J.-C. G. Bünzli, in *Handbook on the Physics and Chemistry of Rare Earths, Volume 37*, 2007, pp. 217–470.

2 V. Ntziachristos, C. Bremer and R. Weissleder, *Eur. Radiol.*, 2003, **13**, 195–208.

3 T. Zhang, X. Zhu, C. C. W. Cheng, W.-M. Kwok, H.-L. Tam, J. Hao, D. W. J. Kwong, W.-K. Wong and K.-L. Wong, *J. Am. Chem. Soc.*, 2011, **133**, 20120–20122.

4 Y. Ning, J. Tang, Y. W. Liu, J. Jing, Y. Sun and J. L. Zhang, *Chem. Sci.*, 2018, **9**, 3742–3753.

5 J. Y. Hu, Y. Ning, Y. S. Meng, J. Zhang, Z. Y. Wu, S. Gao and J. L. Zhang, *Chem. Sci.*, 2017, **8**, 2702–2709.

6 A. D'Aléo, A. Bourdolle, S. Brustlein, T. Fauquier, A. Grichine, A. Duperray, P. L. Baldeck, C. Andraud, S. Brasselet and O. Maury, *Angew. Chemie - Int. Ed.*, 2012, **51**, 6622–6625.

7 S. Comby, S. A. Tuck, L. K. Truman, O. Kotova and T. Gunnlaugsson, *Inorg. Chem.*, 2012, **51**, 10158–10168.

8 Y. Ning, Y. W. Liu, Y. S. Meng and J. L. Zhang, *Inorg. Chem.*, 2018, **57**, 1332–1341.

9 O. A. Blackburn, R. M. Edkins, S. Faulkner, A. M. Kenwright, D. Parker, N. J. Rogers and S. Shuvaev, *Dalton. Trans.*, 2016, **45**, 6782–6800.

10 D. G. Smith, B. K. McMahon, R. Pal and D. Parker, *Chem. Commun.*, 2012, **48**, 8520.

11 B. K. McMahon, R. Pal and D. Parker, *Chem. Commun. (Cambridge, U. K.)*, 2013, **49**, 5363–5365.

12 S. Shuvaev, E. A. Suturina, K. Mason and D. Parker, *Chem. Sci.*, 2018, **9**, 2996–3003.

13 A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc. Perkin Trans. 2*, 1999, 493–504.

14 S. Shuvaev, R. Pal and D. Parker, *Chem. Commun.*, 2017, **53**, 6724–6727.

15 S. Datta and M. Halder, *J. Phys. Chem. B*, 2014, **118**, 6071–6085.