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A novel Eu(III)-based luminescent chemosensor: determining pH in a highly acidic environment

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Abstract—The design, synthesis and photophysical evaluation of the Eu(III)-based chemosensor **[Eu·1]**, is described. The sensors show large (ca. 300 fold) pH dependent luminescence enhancements for the Eu(III) lanthanide emission, i.e. it is '*switched on*' in highly acidic media, between pH ca. 1.5 and 3.5 with a pK_a of 2.4 (±0.1) when excited at 350 nm. The fluorescence spectra of **[Eu·1]**, consisting of the quinoline emission, is also pH dependent, where the fluorescence is '*switched on*' at 458 nm, in the pH region of 3–6, but the emission is quenched between pH 1.5 and 3. Both pH-intensity profiles display an '*off–on–off*' bell-shape behaviour similar to that seen for many enzymatic pH dependent processes. © 2001 Elsevier Science Ltd. All rights reserved.

The design and synthesis of luminescent signalling systems that display large differences between their '*off*' (non emissive) and '*on*' (emissive) states is an active area of research within the field of supramolecular chemistry.[1] The development of such simple first generation switches has led to the development of more complex arrays or single molecules that can perform complicated tasks such as mimicking the function of simple logic gate operations[2] or perform simple tasks as molecular level machines.[3] Such switches are also the basis of luminescent chemosensors for the recognition of physiologically important ions and molecules where the recognition gives rise to perturbations of the photophysical properties of the sensors.[4] There are several advantages in the use of such a mode of detection over, for example, electrochemically based systems, since luminescent sensors give rise to non-invasive, real-time and on-line monitoring.[4] This is particularly important for the monitoring of physiologically important species, and several examples have been reported of the use of fluorescent-based chemosensors[5] for the detection of cations,[5,6] anions[7] and neutral molecules.[8] Nevertheless, the use of a short lived fluorescent excited state is a drawback, because fluorescence detection can be seriously affected by auto-fluorescence and light scattering from the physiological environment. To an extent, this can be overcome by using lumophores (reporters) with relatively long lived excited states, permitting easy resolution from the shorter lifetime emissions, and/or using reporters that absorb or emit at long wavelength (large Stokes shifts). We have recently demonstrated that kinetically and thermodynamically stable luminescent Eu(III) and Tb(III) complexes can be used as chemosensors for the detection of cations[9] and anions.[10] Furthermore, we have demonstrated that such complexes can be employed as molecular level devices for mimicking the function of logic gate operations.[11] Herein, the results of a new kinetically robust chemosensor **[Eu·1]**, which shows highly pH dependent Eu(III) luminescence properties permitting the detection of [H^+] in highly acidic media are reported. Examples of chemosensors that operate at such a low pH are rare,[12,13] since the majority of systems to date have been aimed towards physiological pH detection (pH ~6–8).[5] However, it is necessary to develop chemosensors for monitoring strongly acidic media such as those found in the human stomach, for the study of acidic organelles such as lysosomes and endosomes of live cells,[12] and for environmental monitoring of, for example, fresh water. This is especially important since many conventional fluorescent probes employing fluoresceins, coumarines or rhodamines as reporters are not suitable for such detection since their emission is appreciably decreased under acidic conditions.[12]

The **[Eu·1]** sensor is based upon our earlier design of connecting '*antenna–chromophores*' to a macrocyclic unit which can form stable coordination complexes with lanthanide ions.[9,11] Due to the low molar absorptivities of lanthanide ions such as Eu(III) and Tb(III) (ε ~1) the population of the Eu(III) excited state (Eu(III)*) is difficult, but can be achieved indirectly via

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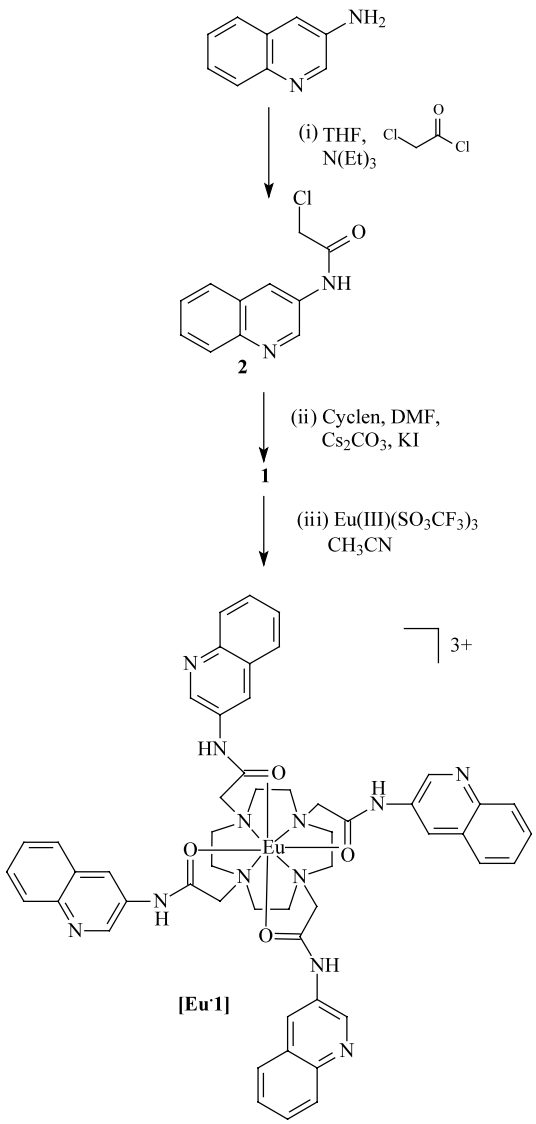
the antenna chromophore which can absorb strongly ($\varepsilon \sim 10^4$), and transfer its S_1 energy via the T_1 state to the Eu(III)*, i.e. via sensitisation.[9,10,14] For **[Eu·1]**, synthesised from 1,4,7,10-tetraazacyclododecane (cyclen), the chemosensor possesses four such antennae, i.e. the four *N*-(3-quinolyl)ethanamide groups, that can be considered as being a combined *antenna–receptor* moiety, since the quinoline nitrogen moiety is a H^+ acceptor. Furthermore, the sensor has a long-lived excited state (~ms range), emits at long wavelengths (580–700 nm), exhibiting large Stokes shifts, and line-like emission bands (ca. 10–30 nm bandwidth) under ambient conditions, all of which are highly desirable features for use in aerobic physiological or environmental monitoring. The synthesis of ligand **1** and the Eu(III)-based chemosensor **[Eu·1]** is shown in Scheme 1. All products were analysed using ^1H and ^{13}C NMR, IR, electrospray (ES) MS, and elementary analysis or accurate mass determination.[15] The α-chloroamide **2** was made in one step by reacting chloroacetyl chloride with 3-aminoquinoline at −10°C in dry THF for 12 h, followed by removal of the inorganic salts by filtration and acid–base extraction, giving an off-white solid, which was then further purified by tituration using CH_2Cl_2, rendering **2** in 65% yield. The ^1H NMR ($CDCl_3$) gave six well-resolved signals for the aromatic protons of **2**, a broad signal at 8.54 ppm for the amide proton and a characteristic singlet at 4.29 ppm for the α-CH_2 protons. The tetra-substituted ligand **1** was formed by reacting 4 equiv. of **2** with cyclen under an inert atmosphere in dry DMF at 80°C for 12 h in the presence of Cs_2CO_3 and KI, followed by acid–base extraction and recrystallisation. The ^1H NMR spectrum of **1** showed multiplet resonances between 8.8 and 7.6 ppm, a broad singlet for the α-methylene spacer ($CONHC\underline{H}_2$), at 4.63 ppm and broad signals between 3.6 and 2.8 ppm for the ring protons of the cyclen. The cationic Eu(III) complex **[Eu·1]** was formed in 40% yield by reacting in dry CH_3CN, the ligand and equimolar quantities of $Eu(CF_3SO_3)_3$ at 80°C, yielding **[Eu·1]** as a fine off-white powder that was collected by centrifugation. The complex was further purified by alumina column chromatography (gradient elution from CH_2Cl_2 to 10% MeOH:CH_2Cl_2). The ^1H NMR of **[Eu·1]** showed characteristic resonances at 17.2, 14.2, −0.5, −2.8, −5.2 and −9.7 for the ring axial, equatorial and the methylene α-protons.



Scheme 1.

The UV–vis absorption, and the fluorescence spectra of **2** and **[Eu·1]** were recorded in water, in the presence of tetramethylammonium perchlorate (2×10^{-2} M) to maintain a constant ionic strength. In alkaline solution (pH 11) the absorption spectrum of **2** gave rise to a strong absorption band at 245 nm with smaller shoulders at 317 and 329 nm. These bands were unchanged in the pH range of 4.7–11. However, upon further acidification they became highly pH dependent, showing hypsochromic shifts with the formation of new bands at 214, 238, 261 and 337 nm and isosbestic points at 231, 243, 253, 273 and 302 nm. From these changes a pK_a of 3.6 (±0.1) was determined, which can be assigned to the protonation of the quinoline nitrogen moiety. The fluorescence emission spectra of **2** were also pH dependent; in alkaline solution two bands of near equal intensity were observed at 356 and 417 nm when excited at 330 nm. However, upon excitation at 302 nm (the isosbestic point) a major band was observed at 356 nm and a smaller band at 417 nm. Upon acidification to below pH 4.5, the intensity of these two bands decreased with the formation of a new broad emission at 400 nm and an isoemissive point at 356 nm. From these changes a pK_a of 3.7 (±0.1) was deduced. The absorption spectrum of **[Eu·1]** was similar to that seen for **2**; with major absorption enhancements observed in the band centred at ca. 330 nm upon acidification below pH 4 (Fig. 1) and a pK_a of 2.3 (±0.1), which is somewhat smaller than seen for **2**. This is most likely due to the presence of the large positively charged lanthanide ion, which makes protonation more difficult. However, since the compound has four possible protonation sites (the four quinoline moieties), it is most likely that each one of them has a slightly different pK_a value from the next. This also explains why the pH switching exceeds the typical two pH units. We have

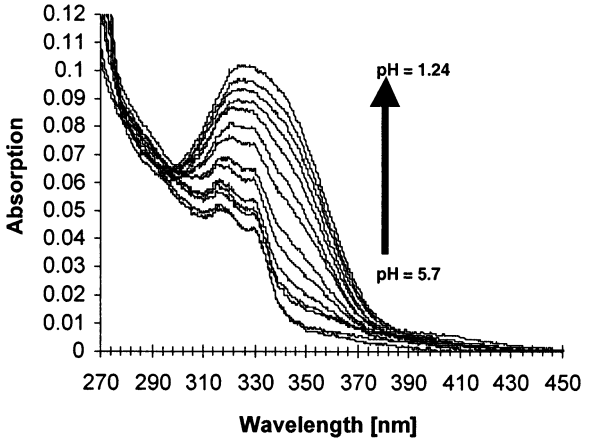


Figure 1. The absorption spectra of **[Eu·1]**, showing the changes upon addition of acid.

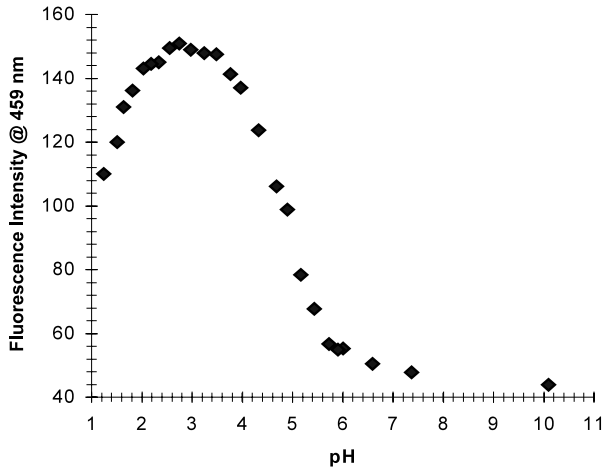


Figure 2. The changes in the fluorescence emission intensity of **[Eu·1]**, when excited at 350 nm, as a function of pH.

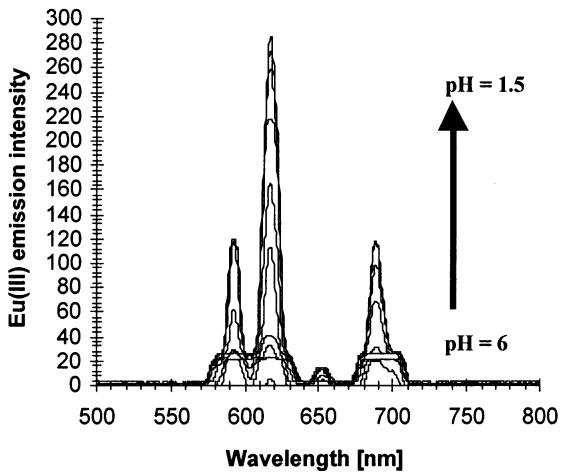


Figure 3. The uncorrected **[Eu·1]** emission spectra, showing the changes upon addition of acid in the pH range 1.5–6. $\lambda_{ex}=350$ nm.

observed similar behaviour for related complexes.[9] Some absorption changes were also observed in alkaline solution, but these changes did not give an accurate pK_a determination. Since large spectral changes occur above 330 nm, the 350 nm wavelength, which shows a large difference in its '*off*' to '*on*' intensity ratio, was chosen for the selective population (i.e. sensitisation) of the Eu(II) excited state, $^5D_0$. This will significantly increase the energy population of the singlet excited state of **[Eu·1]** which in turn transfers its energy to the $^5D_0$ state via the quinoline triplet state, see later. The fluorescence emission spectra of **[Eu·1]** when excited at this wavelength, consisted of a single band centred at 410 nm, when measured in alkaline solution. Upon acidification, the intensity of this band dramatically reduced with the formation of a new band centred at 459 nm, and an isoemissive point at 433 nm. Plotting the changes at 459 nm as a function of pH showed that **[Eu·1]** displays dual fluorescence behaviour (Fig. 2). Upon protonation in the range of pH ca. 6.4 to 3 the fluorescence emission was '*switched on*' with a pK_a of 4.9 (±0.1), whereas below pH 3 the emission was '*switched off*'. This quenching is possibly due to an anion effect. However, it was not possible to accurately determine the pK_a in this strongly acidic medium. The profile is thus displaying '*off–on–off*' fluorescence behaviour, which is centred on pH 3. Such pH profiles have significant implications in biology, since many enzymatic processes take place only within a restricted pH range. **[Eu·1]** can thus be regarded as being a fluorescent sensor for such an acidic pH window. Several similar fluorescent sensors, which display such '*off–on–off*' behaviour, have recently been reported for other pH windows.[16] The rather high pK_a cannot be assigned to the protonation of the nitrogen quinoline moiety, and is thus more likely to be due to the protonation/deprotonation of the quinoline amide nitrogen.[9]

Unlike that seen in the fluorescence emission, the delayed Eu(III) emission was not pH dependent above pH 3.5, when excited at 350 nm (or at 320 nm). In alkaline solution above pH 6 no clear emission enhancement was observed. From pH 6 to 3.5, the complex was only weakly emitting, with the emission intensity remaining almost constant. However, upon titration with acid, below pH 3.5, the Eu(III) emission gradually '*switched on*', and at low pH (~1.5) the **[Eu·1]** showed three well resolved (but broad) emission bands at 592, 616 and 688 nm, and a smaller band at 650 nm, corresponding to the deactivation of the $^5D_0 \rightarrow {}^7F_J$ ($J=1$, 2, 3 and 4) ground states (Fig. 3). These emission enhancements are due to the increased ability of the antenna to transfer its excited state energy to the lanthanide ion (Eu(III)*) in acidic media when excited at 350 nm (Fig. 1). The largest changes were seen in the 616 band ($\Delta J=2$ transition) with a luminescence enhancement of ca. 300 (but in highly alkaline solution, no emission was observed, so the factors could be much larger), and smaller changes in the $\Delta J=1$ and $J=4$ transition. Moreover, the luminescence switching was fully reversible, since addition of strong base (pH ~10) quenches the emission, which could be subsequently '*switched on*' again by the addition of acid (pH 1.6). The lifetime of the Eu(III) excited state was measured to be 767 μs in acid at pH 1.8 when measured in H_2O

but it was not possible to measure the lifetime in alkaline solution. When these measurements were repeated in D_2O, the lifetime was increased to 1231 μs. From these values the Eu(III) hydration state q, the number of bound water molecules was estimated to be 0.52.[17] Due to the nature of the Eu(III), these emission bands have Stokes shifts of ca. 230–340 nm for each of the $^5D_0 \rightarrow {}^7F_J$ bands, when excited at 350 nm. This gives rise to higher signal quality than for analogous fluorescent sensors, which often possess overlapping excitation and emission spectra. Plotting the changes in Eu(II) emission at 616 nm as a function of pH gave a pH-emission profile that had a bell-shaped appearance (Fig. 4). In alkaline solution no emission was observed, but small changes were seen between pH 6 and 4. The largest changes were seen between pH 1.8 and 3.5, which indicates that the Eu(III) emission is signalling the protonation of the nitrogen moiety of the remote quinoline antenna, a feature not as clearly seen in the fluorescence emission spectrum. From these changes a pK_a of 2.4 (±0.1) was determined, which is in good agreement with the changes seen in the absorption spectra. At lower pH the emission is switched partly off, which could possibly be due to anion quenching. We are currently investigating these features further. The '*off–on–off*' switching, in such acidic media has not been demonstrated before using lanthanide-based emitting moieties. In comparison, these Eu(III) emission changes mirror those seen in the absorption spectra, since the sensitisation from the quinoline moiety to the Eu(III)* becomes more feasible in more acidic solution, since the extinction coefficient at 350 nm is gradually increased upon protonation (Fig. 1). The Eu(III) emission can thus be said to be '*switched on*' upon protonation of the quinoline moiety. Thus **[Eu·1]** provides a potential new way of monitoring acidic systems such as organelles.

In summary, the new Eu(III)-based chemosensor **[Eu·1]** displays dual luminescence behaviour; the fluorescence emission is '*switched on*' between pH 3.3 and 5.5; whereas the Eu(III) emission is '*switched on*' between pH 1.8 and 3.5. In both cases the pH profiles are bell shaped, displaying '*off–on–off*' emission behaviour. **[Eu·1]** is the first example of the use of lanthanide-based chemosensors that can detect pH in highly acidic environments.

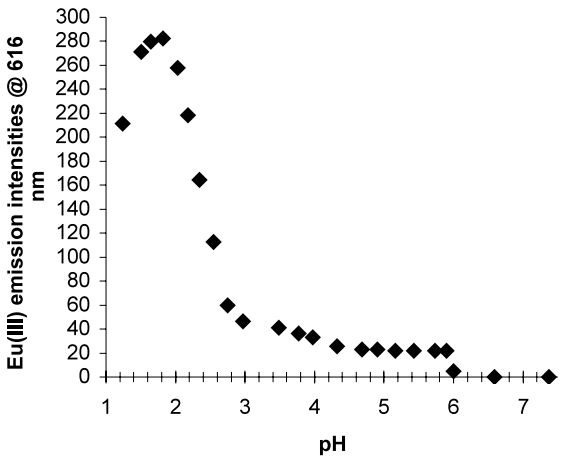


Figure 4. The changes in the Eu(III) emission intensity of **[Eu·1]**, when excited at at 350 nm, as a function of pH.

Acknowledgements

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15. Compound **2**: Anal. calcd for $C_{11}H_9ClN_2O$: C, 59.87; H, 4.11; N, 12.70. Found: C, 59.29; H, 4.04; N, 13.22%. Compound **1**: Calcd for $C_{52}H_{52}N_{12}O_4$: 908.4234 (M^+); found: 908.4230 (M^+). **[Eu·1]**: Calcd for $C_{52}H_{52}EuN_{12}O_4$: 1061.3447 (M^+); found: 1061.3450. δ_H (CD_3OD, 400 MHz): 17.2, 14.2, –0.5, –2.8, –5.2 and –9.7. m/z (% ES^+): 530.32 ($100M^+/2$), 604.16 ([$87M+CF_3SO_3$]/2).
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