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Converting an intensity-based sensor to a ratiometric sensor: luminescence colour switching of an Ir/Eu dyad upon binding of a V-series chemical warfare agent simulant.

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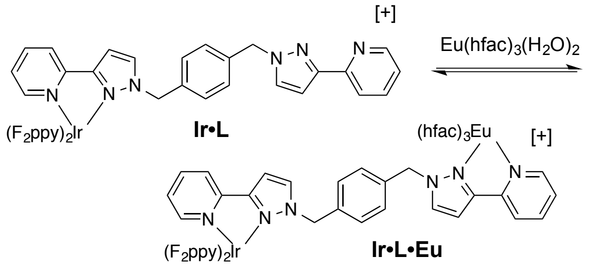
Interaction of the V-series chemical warfare agent simulant 'VO' with Eu(III) results in selective quenching of the red Eu-based emission component from a dual-luminescent (blue/red) Ir(III)/Eu(III) dyad, resulting in a colour change in the luminescence from red to blue in the presence of the simulant.

Trivalent lanthanide ions with strong luminescence in the visible region are commonly used in luminescent sensors and probes due to the favourable combination of narrow emission lines, high intensity and long lifetimes for the emission, and substantial Stokes shifts.[1-4] Sensing of substrates is based on modulation of the metal centered luminescence of the trivalent lanthanide ion. This might be an increase in luminescence intensity if water ligands (which can have a limited quenching effect) are displaced from the coordination sphere of the metal ion;[2] or a decrease in intensity if a sensitising antenna ligand is displaced.[3] In some cases changes in relative intensity between different lines in the Eu(III) emission spectrum have provided a basis for ratiometric sensing.[4] However, analysis of these generally requires spectrophotometric measurements as the spectral changes involved are not always obvious to the naked eye in the form of a clear colour change.

It is therefore desirable to find a way for analyte binding to trivalent lanthanide ions to result in an obvious change in luminescence output that is visible to the naked eye. A solution to this, exploited herein, is to combine trivalent lanthanide-based luminescence whose intensity is responsive to a specific analyte, with the luminescence output from another luminophore in a different region of the spectrum, which is insensitive to the analyte. This allows a change in intensity of one component to be transduced to a much more easily detectable change in colour from the bichromophoric pair. Such a principle has been used in dual-luminescent systems for ratiometric sensing of a range of simple analytes.[5]

We report here the use of an Ir(III)/Eu(III) dyad as the basis of a ratiometric sensor for a chemical warfare agent (CWA) simulant. Strategies for the detection, analysis and destruction of CWAs are of obvious importance,[6] and several examples of luminescence-based sensors have been reported based on a change in luminescence intensity of the probe when a CWA (or a simulant) binds.[2,7] Our ratiometric sensor is the dyad Ir•L•Eu (Scheme 1) which combines a blue-luminescent Ir(III) terminus based on cyclometallated phenylpyridine ligands, with a red-luminescent {Eu(hfac)$_3$} terminus (hfac = anion of hexafluoro-acetylacetone); the two are connected by a bis(pyrazolyl-pyridine) bridging ligand (Scheme 1).[8]



Scheme 1. The equilibrium involved in formation of Ir•L•Eu [F$_2$ppy = 2-(2,4-difluorophenyl)pyridine]

In this dyad, Ir(III)→Eu(III) photoinduced energy-transfer through the conjugated ligand backbone results in partial quenching of the Ir(III)-based emission, with concomitant sensitisation of the Eu(III)-based emission signal.[8,9] As Eu(hfac)$_3$(H$_2$O)$_2$ is added to a solution of Ir•L to generate Ir•L•Eu by the equilibrium shown in Scheme 1, the overall luminescence colour steadily shifts from intense blue (from Ir•L at the start) to intense red (from Ir•L•Eu at the end of the titration). During the titration the luminescence passes through pure white due to a balanced mixture of blue from Ir•L and red from Ir•L•Eu.[8] It follows that anything that can selectively quench the red Eu-based emission component

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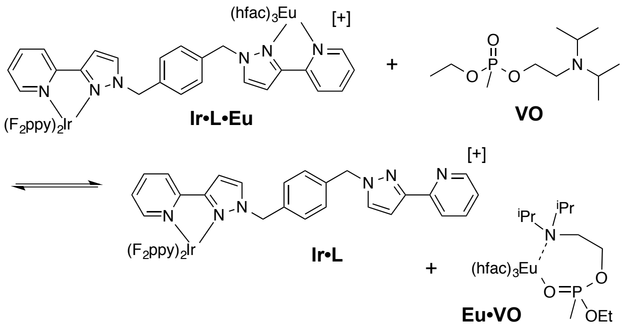
Electronic Supplementary Information (ESI) available: [additional figures and experimental details]. See DOI: 10.1039/x0xx00000x should cause a steady shift in the luminescence colour from red, through white, back to blue as the red/blue balance shifts in favour of the remaining blue emission component.

The analyte we have investigated is the low toxicity V-series organophosphorus chemical warfare agent (OP CWA) simulant 2-diisopropylaminoethyl ethyl methylphosphonate (‘VO’, Scheme 2).[10] The V-series OP CWAs comprise phosphonothioates such as VX, VE and VM and the phosphorothioate VG (amiton) which are structurally different from the well-known G-series OP CWAs such as sarin (GB) and tabun (GA) (ESI Fig. S1), containing characteristic phosphorus sulfur bonds and terminal tertiary amine moieties. The V-series OP CWAs are extremely potent and fast acting acetylcholinesterase inhibitors and are amongst the most toxic substances ever synthesised on a large scale;[10] hence, their accurate, rapid and selective detection is of paramount importance. In recent work Dennison *et al.* investigated the interaction of live V- and G-series OP CWAs with lanthanide(III) ions and the resulting luminescence changes,[11] which we exploit in Ir•L•Eu.



Scheme 2. Interaction of the analyte VO with the sensor Ir•L•Eu [F$_2$ppy = 2-(2,4-difluorophenyl)pyridine]. In the species **Eu•VO** the extent of any dative interaction from the amine of VO to the the Eu(III) centre is unclear (see main text) so this is drawn with a dashed line.

VO can coordinate to lanthanides in a bidentate chelating manner which correlates closely with the binding behaviours observed with the V-series OP CWAs VX and VG.[10] This binding is based primarily on the strength of the P=O•••Ln(III) coordinate bond with quenching of the Eu(III) luminescence being driven by the tertiary amine group, most likely *via* amine→Eu(III) photoinduced electron-transfer.[12] The photo-physical response is complex, with the quenching of Eu(III) luminescence by VO having both static (from the coordinated VO ligand) and dynamic (collisional quenching from excess VO in solution) components.[10] We therefore expect that VO will be able to displace the {Eu(hfac)$_3$} unit from Ir•L•Eu according to Scheme 2, quenching the red luminescence component which is sensitised by Ir→Eu energy-transfer.

Initial control experiments to verify this were performed to probe the interaction with a {Eu(hfac)$_3$} unit of (i) the monodentate O-donor dimethyl methylphosphonate (DMMP, which coordinates through the P=O oxygen atom and is a model for the phosphonate terminus of VO); (ii) Et$_3$N, a model for the tertiary amine terminus of VO; and finally (iii) VO itself, all in MeCN solution. Titration of DMMP into a solution of Eu(hfac)$_3$(H$_2$O)$_2$ (8.3 x 10^{-5} M) resulted in a steady increase in intensity of Eu-based luminescence (Fig. 1a). This is the result of DMMP molecules coordinating to the Eu(III) centre and displacing the water ligands, thereby removing the quenching effect of O–H oscillators. The data could be fitted to a binding model in which the two binding interactions are equivalent with each individual binding constant being *ca.* 49 M^{-1} giving an overall equilibrium constant of 2400 M^{-2} for formation of Eu(hfac)$_3$(DMMP)$_2$ (see ESI for tabulated data).

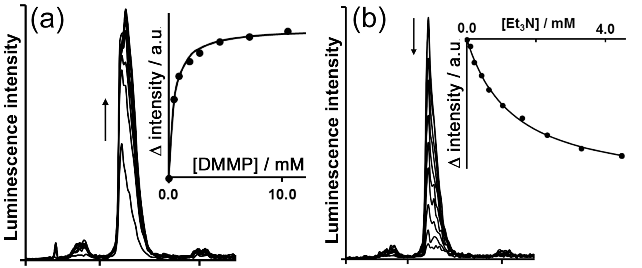


Fig. 1. Change in the luminescence intensity of [Eu(hfac)$_3$(H$_2$O)$_2$] (8.3 x 10^{-5} M) on titration with (a) DMMP in MeCN and (b) Et$_3$N in MeCN (λ_{exc} = 340 nm).

An analogous titration of Et$_3$N into Eu(hfac)$_3$(H$_2$O)$_2$ generated a steady *decrease* in Eu(III) luminescence, consistent with the amine acting as a quencher of the Eu(III) excited state (Fig. 1b). The luminescence quenching curve could be fitted to a 1:1 binding isotherm with K = 780 M^{-1}. This does not, however, correspond solely to formation of a 1:1 complex with coordinate bond formation as (i) Et$_3$N should be a much poorer ligand than water for Eu(III) based on both simple steric and electronic considerations, and (ii) a Stern-Volmer plot showed pronounced upward curvature characteristic of a combination of both static and dynamic quenching (ESI, Fig. S2). We suggest therefore that association of Eu(hfac)$_3$(H$_2$O)$_2$ with Et$_3$N (possibly by weak coordinate bond formation, or by hydrogen-bonding) is responsible for the static quenching, with additional collisional quenching with free Et$_3$N providing the dynamic component. Previous observations confirm that other amine ligands bound only weakly to a Eu(III) centre.[10,11]

The luminescence intensity changes that occurred on titration of VO into Eu(hfac)$_3$(H$_2$O)$_2$ (7.5 x 10^{-5} M) in MeCN (Fig. 2a) demonstrated a combination of the two types of behaviour shown above with DMMP and Et$_3$N. Initially there was a rapid increase in luminescence intensity up to addition of *ca.* 1 equivalent of VO, followed by a much more gradual decay in intensity as excess VO was added. The sharp initial rise in intensity arises from binding of VO which, like other bidentate ligands, can displace the two water molecules from the coordination sphere of Eu(III) to give Eu(hfac)$_3$(VO) with K = 7 x 10^4 M^{-1}. We note that the bidentate chelating behaviour of neutral VO is not strong enough to displace the tightly bound, anionic, hfac ligands. Whilst the proximity of the amine to Eu(III) is expected to cause quenching (*cf.* the titration with Et$_3$N), displacement of the two water ligands from Eu(III) is expected to increase luminescence (*cf.* the titration with DMMP), and the balance of the two effects results in a net luminescence intensity increase when VO binds (Fig. 2a). The subsequent decrease in luminescence intensity as excess VO is added (which can be fitted to a 1:1 isotherm with $K = 640$ M^{-1}) is strikingly similar to the behaviour that we observed when Et_3N was added, with a Stern-Volmer plot showing non-linear behaviour from an obvious mixture of static and dynamic quenching components (Fig. 2b). Overall, the picture that emerges from these experiments is that chelating coordination of VO to a {Eu(hfac)$_3$} unit is principally driven by the 'hard' phosphonate O-donor, with the more weakly coordinating amine group providing quenching of Eu(III) luminescence.

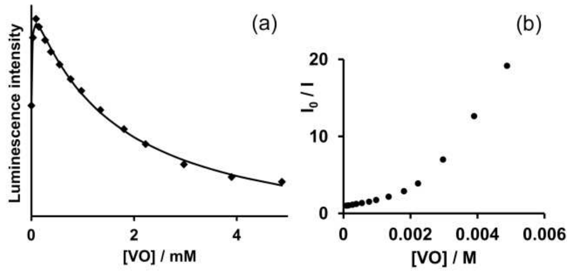


Fig. 2. (a) Change in the luminescence intensity of [Eu(hfac)$_3$(H$_2$O)$_2$] (7.5 x 10^{-5} M) on titration with VO in MeCN (λ_{exc} = 340 nm), based on integration of the 615 nm emission line; note the initial rise (first three data points) and then slower decay. (b) A Stern-Volmer plot of the same data (starting from the third point where the decay commences), showing the upward curvature characteristic of a mixture of static and dynamic quenching.

With this understanding we investigated next the behaviour of Ir•L•Eu as a ratiometric sensor for VO. Ir•L•Eu is formed according to the equilibrium shown in Scheme 1 from titration of Eu(hfac)$_3$(H$_2$O)$_2$ into a solution of Ir•L in MeCN, resulting in partial quenching of Ir-based luminescence (λ_{max} = 455 nm) and the appearance of sensitised Eu-based emission arising from Ir→Eu energy-transfer (Fig. 3).[10]

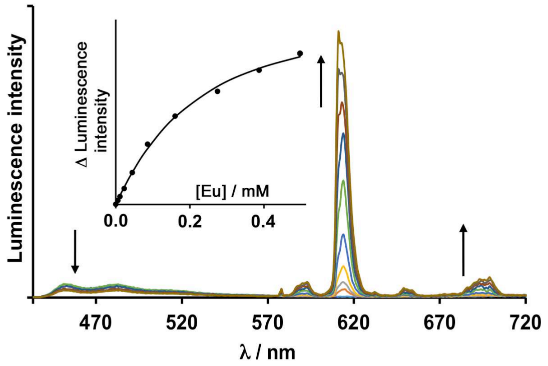


Fig. 3. Changes in the luminescence spectra (λ_{exc} = 395 nm) associated with formation of Ir•L•Eu from Ir•L (10^{-4} M) and Eu(hfac)$_3$(H$_2$O)$_2$ according to Scheme 1, with the blue Ir-based emission being slightly quenched and sensitised Eu-based emission growing in as the dyad forms. Inset: integrated intensity of the 615 nm Eu-based emission signal during the titration, fit to a 1:1 binding isotherm.

Importantly, the excitation wavelength used for luminescence measurements on Ir•L•Eu is 395 nm – a region where the Ir chromophore absorbs, but free Eu(hfac)$_3$(H$_2$O)$_2$ and Eu(hfac)$_3$(VO) do not. Thus, in the evolving luminescence spectra, the *only* Eu-based emission that is present is sensitised emission from Ir→Eu energy-transfer in the intact dyad; Eu(hfac)$_3$(H$_2$O)$_2$ and Eu(hfac)$_3$(VO) are effectively invisible. From the rise in intensity of sensitised Eu-based emission as Ir•L•Eu forms during this titration it was determined that the equilibrium constant for the reaction in Scheme 1 is 6800 M^{-1}. Stock solutions of Ir•L•Eu were prepared typically containing *ca.* 10^{-4} M Ir•L and 1.1 equiv. Eu(hfac)$_3$(H$_2$O)$_2$ in MeCN, such that the equilibrium in Scheme 1 lies to the right with the concentration of Ir•L•Eu being typically 8 x 10^{-5} M. Titration of VO into this solution resulted in progressive quenching of the sensitised Eu(III) metal-based emission following excitation of the Ir(III) unit at 395 nm (Fig. 4). We recall from the control measurements that VO has a higher affinity for the {Eu(hfac)$_3$} unit ($K \approx 7 \times 10^4$ M^{-1}) than does the pyrazolyl-pyridine unit ($K \approx 7 \times 10^3$ M^{-1}): so it is to be expected that VO will extract the {Eu(hfac)$_3$} unit from the Ir•L•Eu dyad to form [Eu(hfac)$_3$(VO)] which can no longer be sensitised by energy-transfer from the Ir(III) unit. The Ir(III)-based emission component scarcely changes as the primary coordination sphere of the kinetically inert Ir(III) unit does not change when VO is added.

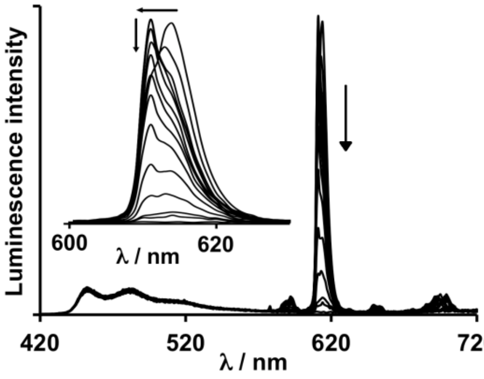


Fig. 4. Quenching of the Eu-based emission component of Ir•L•Eu [prepared from a mixture of 10^{-4} M Ir•L and 1.1 equiv. Eu(hfac)$_3$(H$_2$O)$_2$, see main text] during titration with VO in MeCN (λ_{exc} = 395 nm). Inset: expansion of the 615 nm Eu-based emission line during the titration.

The reduction in sensitised Eu(III)-based emission intensity during the titration of Ir•L•Eu with VO can be fitted to a 1:1 binding isotherm to generate a binding constant of 410 M^{-1}, similar to the values obtained for the Eu(hfac)$_3$(H$_2$O)$_2$/Et_3N and Eu(hfac)$_3$(H$_2$O)$_2$/VO control experiments (see above). The ratio of the two (Ir-based and Eu-based) emission intensities during the titration is shown in the ESI (Fig. S3).

After *ca.* 8 equivalents of VO were added the red luminescence of the solution under a hand-held UV lamp has faded and taken on a purple cast; after about 30 equivalents the luminescence appeared white; and thereafter the blue Ir(III)-based luminescence dominated (Fig. 5a). The change in colour based on the luminescence spectra can be conveniently indicated on a CIE chromaticity diagram (ESI, Fig. S4). The quenching of the Eu(III)-based luminescence during the titration will arise from two factors: (i) formation of Eu(hfac)$_3$(VO) as the VO ligand removes the {Eu(hfac)$_3$} unit from the dyad, so that its emission can no longer be sensitised by the Ir unit; and (ii) additional dynamic quenching of any intact Ir•L•Eu by the excess VO free in solution.[11] In agreement with this, we note that the quenching constant of 410 M^{-1} from the data in Fig. 4 is at least an order of magnitude greater than would be expected based on coordination of VO to the {Eu(hfac)$_3$} unit alone. Since binding of {Eu(hfac)$_3$} to the pyrazolyl-pyridine chelate and to VO have *K* values of *ca.* 7 x 10^3 M^{-1} and 7 x 10^4 M^{-1} respectively, Scheme 2 should have $K \approx 10^1$ M^{-1}. The value of 410 M^{-1} derived from quenching of Eu(III) luminescence in Fig. 4 implies that the quenching has a substantial dynamic component above and beyond quenching caused by direct complex formation with VO.

Importantly, dynamic quenching of Ir(III)-based blue luminescence by the amine component of VO is insignificant compared to the dynamic quenching of Eu-based emission, because the luminescence lifetime of the Ir unit is three orders of magnitude shorter than that of the Eu unit. Thus the VO has a strong effect on quenching Eu-based emission – both removal from the dyad by chelation and by dynamic quenching – whereas it has very little effect on the Ir-based emission. The result is a change in the red:blue balance during the titration which provides the observed colour change.

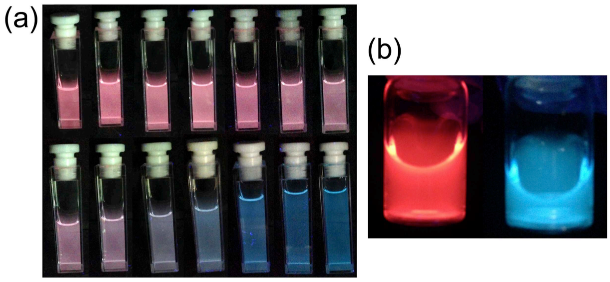


Fig. 5. (a) Colour changes in the luminescence observed from Ir•L•Eu [prepared from a mixture of 10^{-4} M Ir•L and 1.1 equiv. Eu(hfac)$_3$(H$_2$O)$_2$ in MeCN, see main text] during titration with VO in MeCN using a hand-held UV lamp (λ_{exc} = 365 nm) as the excitation source. (b) Colour change response of luminescence from Ir•L•Eu (*ca.* 3 x 10^{-4} M, see main text) before (left) and after (right) addition of 18 equiv. of VO in MeCN.

Use of higher concentrations make the colour changes in the luminescence particularly obvious. Fig. 5(b) shows a solution prepared using Ir•L (3.85 x 10^{-4} M) and Eu(hfac)$_3$(H$_2$O)$_2$ (1.14 x 10^{-4} M); the excess of the Ir component ensures that there is little free Eu(hfac)$_3$(H$_2$O)$_2$ at the start of the titration, with the concentration of Ir•L•Eu being 3 x 10^{-4} M based on the equilibrium constant. The change in luminescence colour from intense red to intense blue under a hand-held UV lamp in the presence of 18 equivalents of VO is striking.

In conclusion, we have shown that (i) selectively quenching the Eu-based luminescence component in an Ir(III)/Eu(III) dyad allows a change in intensity of the Eu-based emission to be converted to an obvious ratiometric change in overall luminescence colour; and (ii) applied this to demonstrate a ratiometric luminescence response for detection of the V-series CWA simulant VO which is specifically based on its bifunctional nature (phosphonate *O*-donor *and* amine-based quencher). Monodentate *O*-donor CWA simulants (such as DMMP, a simulant for G-series CWAs) do not show the same behaviour. Since VO has been shown recently to provide a photophysical response on coordination to lanthanides that mirrors that of the active V-series OP CWAs,[11] the sensing behaviour that we have demonstrated should likewise translate to active CWAs of this type. More generally, the underlying method provides a promising way to achieve ratiometric detection of the very wide range of substrates that are known to bind to lanthanide(III) ions and cause changes in emission intensity.

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