Introduction

Hydrogen sulphide (H$_2$S) is the smallest bioactive thiol that may act as a gaseous signalling agent, and its production in different tissue types is associated with a wide range of physiological responses such as vascular smooth muscle relaxation, mitochondrial ATP production, insulin-signalling inhibition, regulation of inflammation response and mediation of neurotransmission. Moreover, recent investigations show that abnormal levels of H$_2$S are associated with a variety of diseases, such as neurodegenerative diseases, diabetes and cancer. However, the biological targets of H$_2$S and the mechanisms of these H$_2$S-related physiological phenomena remain unclear. Therefore the development of responsive and reversible luminescence probes for non-invasive real time monitoring of H$_2$S may be useful for understanding its biological modes of action.

One of the major approaches for developing luminescence H$_2$S detection is based on sulphide-specific chemical reactions, such as reduction of an azide and nucleophilic addition of a sulphide ion. This type of luminescence probe is generally irreversible and usually requires a considerably long incubation time. An alternative approach is based on CuS precipitation due to the low-solubility of CuS ($K_{sp} = 6.3 \times 10^{-36}$). These luminescence probes are generally reversible with low detection limits. We are particularly interested in developing H$_2$S luminescence sensors based on organo-lanthanide complexes due to their water-solubility and unique photophysical properties, including line-like emission spectra and long luminescence lifetimes (micro to millisecond scale) that can effectively separate the observing signal from biological autofluorescence noise and are suitable for time-gated detection. Recently, a few studies have been found in the literature with irreversible H$_2$S lanthanide probes. Herein, we report the development of a novel responsive europium-based luminescence “off–on” gate for the in situ detection of H$_2$S in water.

As illustrated in Fig. 1, EuL$1$ contains a DO3A–Eu$^{3+}$ complex and an azathcomposer-6 moiety, which are linked to the 2- and 6-positions of a pyridine-containing chromophore constituting a switch-like structure. In the ground state, EuL$1$ should be emissive due to the coordination of the pyridine chromophore.
to a Eu³⁺ ion, which favours energy transfer from the organic chromophore to the Eu³⁺ ion. Upon binding of the aza-18-crown-6 moiety with a Cu²⁺ ion, pyridine is expected to coordinate with the Cu²⁺ ion, resulting in luminescence quenching. The europium emission should be recovered after the displacement of the Cu²⁺ ion upon copper sulphide precipitation.

Results and discussion

Synthesis and photophysical properties of L1 and EuL1

Ligand L1 was readily prepared from (4-iodopyridine-2,6-diyl)dimethanol (I) via a desymmetrization synthetic strategy. As shown in Scheme 1, a pyridine-containing chromophore (based on a D–p–A motif) was established via a Sonogashira cross-coupling reaction between I and 1-ethynyl-4-propoxybenzene (2). After converting both hydroxyl groups of 3 into the corresponding bromide, the aza-18-crown-6 and DO3A moieties were incorporated into 4 sequentially under basic conditions and afforded L1 in good yields. L1 was fully characterized using ¹H and ¹³C NMR spectroscopy and HRMS. Finally, acid hydrolysis of the t-butyl esters followed by Eu complex formation provided EuL1, which was characterized unambiguously using HRMS and HPLC (Table S1 and Fig. S1†).

In the UV-vis absorption spectrum, L1 showed strong absorption bands at 235 and 310 nm in methanol which are attributed to the π to π* transitions. The absorption bands were broadened and red-shifted in EuL1 (245 and 333 nm, ε₃₃₃ nm = 7560 M⁻¹ cm⁻¹) in water (Fig. S2†). The excitation spectrum of EuL1 at 615 nm showed maxima at 240 and 340 nm (Fig. S2†), evidencing an antenna effect due to energy transfer from the ligand to the Eu³⁺ ion. The ⁵D₀ → ⁷F₇ transitions of EuL1 (λₑₓ = 325 nm) were found at 578 (J = 0), 604–637 (J = 2), 646–658 (J = 3), and 673–712 nm (J = 4) in the emission spectrum (Fig. 2). The quantum yield of EuL1 corresponding to the ⁵D₀ → ⁷F₂ transitions of Eu³⁺ ions in water is 0.5% (Table S2†).

Fluorimetric titration studies of EuL1

With EuL1 in hand, its binding properties towards Cu²⁺ ions were investigated. Upon the addition of 1 equiv. of Cu²⁺ ions (CuCl₂ as the source of Cu²⁺ ions), the absorption maximum of EuL1 showed a slight red shift and the absorption ability slightly decreased due to the effect of the copper metal. In a titration study, EuL1 exhibited a 17-fold quenching of the
europium emission with an excess of Cu²⁺ ions and the Benesi–Hildebrand plot showed a 1 : 1 binding stoichiometry with \( K_B = 1.2 \times 10^5 \text{M}^{-1} \) (inset of Fig. 3a). The Job’s plot also supported the formation of a EuL₁–Cu²⁺ complex in a 1 : 1 ratio (Fig. S3†).

In a competitive study, the addition of a large excess of various metal ions, such as Na⁺, K⁺, Ca²⁺, Mg²⁺, Ba²⁺, Ba²⁺, Co²⁺, Zn²⁺, Ni²⁺, Fe³⁺, Mn²⁺, Cu⁺ and Li⁺ ions, to EuL₁ resulted in only slight luminescence changes (red columns in Fig. 3b). The subsequent addition of excess Cu²⁺ ions caused significant luminescence quenching (blue columns in Fig. 3b). These results indicate the high selectivity of EuL₁ towards Cu²⁺ ions and that the binding between EuL₁ and Cu²⁺ ions is not interfered by other metal ions. In a pH study, EuL₁ remains highly emissive and was quenched by Cu²⁺ ions in the pH range 6 to 8 (Fig. S4†), indicating that EuL₁ is stable and can bind to Cu²⁺ ions under physiological conditions.

To study the reversibility of the binding between EuL₁ and Cu²⁺ ions, a small amount of H₂S (Na₂S as the source of H₂S) was added. The EuL₁–Cu²⁺ complex responded instantaneously (requiring only 40 s to reach saturation without stirring or shaking) (Fig. S5†), and Eu emission resumed with a similar profile for the emission spectrum to that of EuL₁ (Fig. 4). This result indicated that the DO3A–Eu³⁺ complex was not displaced by a Cu²⁺ ion, forming the EuL₁–Cu²⁺ complex in the previous step. More interestingly, Eu emission was further enhanced (40-fold) with an excess of H₂S and the Eu³⁺ emission profile showed significant changes, suggesting binding between EuL₁ and H₂S (Fig. 5a). The Benesi–Hildebrand plot showed a 1 : 1 binding stoichiometry with \( K_B = 1.5 \times 10^7 \text{M}^{-1} \) (inset of Fig. 5a). The detection limit of EuL₁ towards H₂S was calculated according to the 3\( S_D \)/slope as low as 60 nM. Surprisingly, direct titration of EuL₁ against H₂S resulted in only about a 5-fold luminescence enhancement with a non-linear relationship in the 1 : 1 Benesi–Hildebrand plot (Fig. 6). These results indicated that the Cu²⁺ ion facilitates the specific 1 : 1 binding of EuL₁ and H₂S, presumably via pre-organizing the conformation of EuL₁. On the other hand, non-specific binding (possibly a mixture of 1 : 1 and 2 : 1 binding) between EuL₁ and H₂S resulted without the favourable conformation that is induced by
the pre-complexation of a Cu\(^{2+}\) ion. This proposal was further supported by the dramatic luminescence drop of the EuL\(_1\)–Na\(_2\)S complex upon heating (>70 °C) (Fig. S6†). This type of Cu\(^{2+}\)-assisted luminescence enhancement of Eu emission is unprecedented. In a competitive study, EuL\(_1\)–Cu\(^{2+}\) showed insignificant changes in luminescence with a large excess of anions, including Cl\(^-\), SO\(_4\)\(^{2-}\), HSO\(_4\)\(^-\), I\(^-\), CO\(_3\)\(^{2-}\), HPO\(_4\)\(^{2-}\), Br\(^-\) and HCO\(_3\)\(^-\), and only small changes for GSH and cysteine (red columns in Fig. 5b). Upon the addition of H\(_2\)S, the Eu emissions were recovered in all the above cases, indicating a high selectivity of EuL\(_1\)–Cu\(^{2+}\) towards H\(_2\)S.

Mechanistic studies

The binding mechanisms of EuL\(_1\) towards Cu\(^{2+}\) ions and the EuL\(_1\)–Cu\(^{2+}\) complex towards H\(_2\)S were studied using a comparative analysis of the emission spectra of the Eu complexes (\(\lambda_{ex} = 325\) nm). Table 1 lists the ratio of the emission bands of EuL\(_1\), EuL\(_1\)–Cu\(^{2+}\) and EuL\(_1\)–Cu\(^{2+}\) + H\(_2\)S.

| Compound          | \(5\)D\(0\) \(\rightarrow\) \(7\)F\(_0\) | \(7\)F\(_1\) | \(7\)F\(_2\) | \(7\)F\(_3\) | \(7\)F\(_4\) |
|------------------|------------------|---------|---------|---------|---------|
| EuL\(_1\)        | 0.01             | 1       | 1.22    | 0.08    | 0.35    |
| EuL\(_1\)–Cu\(^{2+}\) | 0.08             | 1       | 1.86    | 0.15    | 0.91    |
| EuL\(_1\)–Cu\(^{2+}\) + H\(_2\)S | 0.48             | 1       | 3.98    | 0.15    | 1.95    |

\(a\) All spectra were acquired in water with excitation at 325 nm.

![Fig. 7 Top: proposed binding mechanism of EuL\(_1\) towards Cu\(^{2+}\) and H\(_2\)S (Na\(_2\)S as the source of H\(_2\)S). Bottom left: emission spectra of the Eu complexes (\(\lambda_{ex} = 325\) nm). Bottom right: \(^1\)H NMR spectra of the La complexes (6.5–8.5 ppm).](https://example.com/fig7)

![Fig. 8 The structures of the negative control compounds EuL\(_2\), EuL\(_3\), L4 and L5.](https://example.com/fig8)
aza-18-crown-6–Cu²⁺ complexes, causing significant luminescence quenching. Moreover, the binding of Cu²⁺ would also provide a favourable conformation for forming a new 1 : 1 complex with H₂S. Upon the addition of H₂S, the emission profile of EuL₁ changed significantly, $\Delta\lambda = 2/\Delta\lambda = 1$ for [EuL₁ + Cu²⁺ + H₂S] and the intensity ratio was about >200% higher for [EuL₁] and [EuL₁ + Cu²⁺]. This increase can be attributed to the lower symmetry of the complexes with the addition of sulphide ions (Fig. 7) and the $^1$H NMR signals of LaL₁ were sharpened. These results suggested new complex formation after the displacement of the Cu²⁺ ion via CuS precipitation. This proposal is further supported by the HRMS spectrum of the EuL₁–Na₂S complex (Fig. S7†) and the change in the quantum yields (Table S2†). The EuL₁–Na₂S complex is highly emissive probably due to its rigid structure.

The proposed binding mechanism was also examined using a series of negative control compounds (Fig. 8). EuL₂ showed no luminescence quenching upon the addition of Cu²⁺ ions (Fig. 9a). This result indicated that the carbonyl linker of aza-18-crown-6 may be too rigid for coordination between Cu²⁺ and pyridine, which could be essential for Eu emission quenching. Without the aza-crown moiety, EuL₃ also showed no luminescence quenching towards Cu²⁺ (Fig. 9b), suggesting DO3A–Eu³⁺ is stable with Cu²⁺ and the aza-crown motif is important for the Cu²⁺ binding. L₄ bearing the pyridine-chromophore showed profound luminescence quenching, but its phenyl analogue (L₅) showed no significant change in luminescence upon the addition of Cu²⁺ ions (Fig. 9c and d). These results indicated that the pyridine moiety of the chromophore is essential for the binding of Cu²⁺ to the aza-crown moiety. The results of this series of negative control compounds are in full agreement with the proposed mechanism in Fig. 7.

Conclusions

In summary, we have prepared a water-soluble and emissive Eu-complex (EuL₁) based on a DO3A(Eu³⁺)–pyridine–aza-crown motif, and studied its consecutive binding properties towards Cu²⁺ and H₂S extensively. EuL₁ binds to Cu²⁺ ions selectively ($K_B = 1.2 \times 10^7 M^{-1}$) inducing 17-fold luminescence quenching and forming a 1 : 1 stoichiometric complex (EuL₁–Cu²⁺), which responds to H₂S selectively with restoration of the original EuL₁ emission followed by a further 40-fold luminescence enhancement and a nano-molar detection limit (60 nM). Mass spectroscopic analysis showed the formation of a 1 : 1 stoichiometric complex (EuL₁–Na₂S) with $K_B = 1.5 \times 10^4 M^{-1}$. Without Cu²⁺ ions, EuL₁ shows non-specific binding towards H₂S with only a 5-fold luminescence enhancement. These results indicate that the Cu²⁺ ion may pre-organize the conformation of EuL₁ and facilitate the formation of the EuL₁–Na₂S complex. The studies
on this unprecedented Cu\textsuperscript{2+}-assisted luminescence enhancement of Eu emission are still ongoing. With long-lived Eu emission, reversible binding properties, an instantaneous response and high selectivity towards H\textsubscript{2}S, this Eu-based luminescence “off-on” gate could find suitable applications for H\textsubscript{2}S imaging in biological systems.

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Notes and references

1 (a) B. Olas, *Clin. Chim. Acta*, 2015, 439, 212; (b) H. Kimura, *Antioxid. Redox Signalging*, 2014, 20, 783; (c) H. Kimura, N. Shibuya and Y. Kimura, *Antioxid. Redox Signalging*, 2012, 17, 45; (d) C. Szabó, *Nat. Rev. Drug Discovery*, 2007, 6, 917.

2 G. D. Yang, L. Y. Wu, B. Jiang, W. Yang, J. S. Qi, K. Cao, Q. H. Meng, A. K. Mustafa, W. T. Mu, S. M. Zhang, S. H. Snyder and R. Wang, *Science*, 2008, 322, 587.

3 (a) M. Fu, W. Zhang, L. Wu, G. Yang, H. Li and R. Wang, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, 109, 2943; (b) G. A. Benavides, G. L. Squadrito, R. W. Mills, H. D. Patel, T. S. Isbell, R. P. Patel, V. M. Darley-Usmar, J. E. Doeller and D. W. Kraus, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, 104, 17977.

4 (a) Y. Kaneko, Y. Kimura, H. Kimura and I. Niki, *Diabetes*, 2006, 55, 1391; (b) W. Yang, G. D. Yang, X. M. Jia, L. Y. Wu and R. Wang, *J. Physiol.*, 2005, 569, 519.

5 (a) Y. J. Peng, J. Naduri, G. Raghuraman, D. Souvannakitti, M. M. Gadalla, G. K. Kadam, S. H. Snyder and N. R. Prabhakar, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, 107, 10719; (b) L. Li, M. Bhattacharyya, Y. Z. Zhu, Y. C. Zhu, R. D. Ramnath, Z. J. Wang, F. B. M. Anuar, M. Whiteman, M. Salto-Tellez and P. K. Moore, *FASEB J.*, 2005, 19, 1196.

6 K. Abe and H. J. Kimura, *J. Neurosci.*, 1996, 16, 1066.

7 (a) B. D. Paul, J. I. Sbodio, R. Xu, M. S. Vandiver, J. Y. Cha, A. M. Snowman and S. H. Snyder, *Nature*, 2014, 509, 96; (b) L. F. Hu, M. Lu, C. X. Tiong, G. S. Dawe, G. Hu and J. S. Bian, *Aging Cell*, 2010, 9, 135; (c) D. Giuliani, A. Ottani, D. Zaffe, M. Galantucci, F. Strinati, R. Lodi and S. Guarini, *Neurobiol. Learn. Mem.*, 2013, 104, 82.

8 (a) L. Wu, W. Yang, X. Jia, G. Yang, D. Duridanova, K. Cao and R. Wang, *Lab. Invest.*, 2009, 89, 59; (b) W. Yang, G. Yang, X. Jia, L. Wu and R. Wang, *J. Physiol.*, 2005, 569, 519.

9 (a) J. Huang, S. Kumar, N. Abbassi-Ghad, P. Španěl, D. Smith and G. B. Hanna, *Anal. Chem.*, 2013, 85, 3409; (b) C. Szabó, C. Coletta, C. Chao, K. Módis, B. Szczesny, A. Papapetropoulos and M. R. Hellmich, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, 110, 12474.

10 For reviews, see: (a) V. S. Lin, W. Chen, M. Xian and C. J. Chang, *Chem. Soc. Rev.*, 2015, 44, 4596; (b) E. L. Que, D. W. Domaille and C. J. Chang, *Chem. Rev.*, 2008, 108, 1517.

11 For selected examples, see: (a) M. Tropiano and S. Faulkner, *Chem. Commun.*, 2014, 50, 4696; (b) V. S. Lin, A. R. Lippert and C. J. Chang, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, 110, 7131; (c) S. Chen, Z.-J. Chen, W. Ren and H.-W. Al, *J. Am. Chem. Soc.*, 2012, 134, 9589; (d) A. R. Lippert, E. J. New and C. J. Chang, *J. Am. Chem. Soc.*, 2011, 133, 10078; (e) H. Peng, Y. Cheng, C. Dai, A. L. King, B. L. Predmore, D. J. Lefer and B. A. Wang, *Angew. Chem., Int. Ed.*, 2011, 50, 9672.

12 For selected examples, see: (a) J. Cao, R. Lopez, J. M. Thacker, J. Y. Moon, C. Jiang, S. N. S. Morris, J. H. Bauer, P. Tao, R. P. Mason and A. R. Lippert, *Chem. Sci.*, 2015, 6, 1979; (b) Z. Huang, S. Ding, D. Yu, F. Huang and G. Feng, *Chem. Commun.*, 2014, 50, 9185; (c) X. Li, S. Zhang, J. Cao, N. Xie, T. Liu, B. Yang, Q. He and Y. Hu, *Chem. Commun.*, 2013, 49, 8656; (d) Y. Qian, L. Zhang, S. Ding, X. Deng, C. He, X. E. Zheng, H.-L. Zhu and J. Zhao, *Chem. Sci.*, 2012, 3, 2920; (e) Y. Qian, J. Karpus, O. Kabili, S.-Y. Zhang, H.-L. Zhu, R. Banerjee, J. Zhao and C. He, *Nat. Commun.*, 2011, 2, 495.

13 For selected examples, see: (a) L. E. Santos-Figueroa, C. de la Torre, S. El Sayed, F. Sancenón, R. Martínez-Máñez, A. M. Costero, S. Gil and M. Parra, *Eur. J. Inorg. Chem.*, 2014, 41; (b) X. Qu, C. Li, H. Chen, J. Mack, Z. Guo and Z. Shen, *Chem. Commun.*, 2013, 49, 7510; (c) M.-Q. Wang, K. Li, J.-T. Hou, M.-Y. Wu, Z. Huang and X.-Q. Yu, *J. Org. Chem.*, 2012, 77, 8350; (d) F. Hou, J. Cheng, P. Xi, F. Chen, L. Huang, G. Xie, Y. Shi, H. Liu, D. Bai and Z. Zeng, *Dalton Trans.*, 2012, 41, 5799; (e) F. Hou, L. Huang, P. Xi, J. Cheng, X. Zhao, G. Xie, Y. Shi, F. Cheng, X. Yao, D. Bai and Z. Zeng, *Inorg. Chem.*, 2012, 51, 2454; (f) K. Sasakura, K. Hanaoka, N. Shibuya, Y. Mikami, Y. Kimura, T. Komatsu, T. Ueno, T. Terai, H. Kimura and T. Nagano, *J. Am. Chem. Soc.*, 2011, 133, 18003.

14 L. C. Gilday, T. Lang, A. Caballero, P. J. Costa, V. Flix and P. D. Beer, *Angew. Chem., Int. Ed.*, 2013, 52, 4356.

15 K. Sonogashira, Y. Tohdia and N. Hagiwara, *Tetrahedron Lett.*, 1975, 16, 4467.

16 (a) H. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, 71, 2703–2707; (b) K. A. Connors, *Binding constants: the measurement of molecular complex stability*, Wiley, New York, 1987.

17 The preparation and characterization of Lat1 are available in the ESL.

18 J.-C. G. Bünzli and G.-O. Pradervand, *J. Chem. Phys.*, 1986, 85, 2489.

19 The synthesis and characterization of the negative control compounds [EuL2, EuL3, L4 and L5] are available in the ESL.