A smart “off–on” gate for the in situ detection of hydrogen sulphide with Cu(II)-assisted europium emission†

Zhenhao Liang,ab Tik-Hung Tsoi,bi Chi-Fai Chan,c Lixiong Dai,bc Yudan Wu,a Guangyan Du,a Lizhi Zhu,ab Chi-Sing Lee,xa Wing-Tak Wong,xb Ga-Lai Lawxb and Ka-Leung Wongxc

A water-soluble and emissive Eu-complex (EuL1) bearing a DO3A(Eu3+)–pyridine–aza-crown motif has been prepared and its Cu2+ complex has been demonstrated to be a smart luminescence “off–on” gate for H2S detection in water with a nano-molar detection limit (60 nM). EuL1 binds to Cu2+ ions selectively (KfiB = 1.2 × 105 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 Eu emission of EuL1 followed by a further 40-fold luminescence enhancement, forming a 1 : 1 stoichiometric complex (EuL1–Na2S, KfiB = 1.5 × 104 M–1). Without Cu2+ ions, EuL1 showed non-specific binding towards H2S with only a 5-fold luminescence enhancement.

Introduction

Hydrogen sulphide (H2S) 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 H2S are associated with a variety of diseases, such as neurodegenerative diseases, diabetes and cancer. However, the biological targets of H2S and the mechanisms of these H2S-related physiological phenomena remain unclear. Therefore the development of responsive and reversible luminescence probes for non-invasive real time monitoring of H2S may be useful for understanding its biological modes of action.

One of the major approaches for developing luminescence H2S 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 (Ksp = 6.3 × 10–36). These luminescence probes are generally reversible with low detection limits. We are particularly interested in developing H2S 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 milli second 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 H2S lanthanide probes.

Herein, we report the development of a novel responsive europium-based luminescence “off–on” gate for the in situ detection of H2S in water.

As illustrated in Fig. 1, EuL1 contains a DO3A–Eu3+ complex and an aza-18-crown-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, EuL1 should be emissive due to the coordination of the pyridine chromophore

† Electronic supplementary information (ESI) available: Detailed experimental procedures, characterization of compounds, NMR spectra and supplementary fluorometric titration studies. See DOI: 10.1039/c5sc04091d

Received 28th October 2015 Accepted 7th December 2015 DOI: 10.1039/c5sc04091d www.rsc.org/chemicalscience
to a Eu\(^{3+}\) ion, which favours energy transfer from the organic chromophore to the Eu\(^{3+}\) ion. Upon binding of the aza-18-crown-6 moiety with a Cu\(^{2+}\) ion, pyridine is expected to coordinate with the Cu\(^{2+}\) ion, resulting in luminescence quenching. The europium emission should be recovered after the displacement of the Cu\(^{2+}\) 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 (1) 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 1 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 \(^1\)H and \(^13\)C NMR spectroscopy and HRMS. Finally, acid hydrolysis of the \(\tau\)-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 \(\pi\) to \(\pi^*\) transitions. The absorption bands were broadened and red-shifted in EuL1 (245 and 333 nm, \(\epsilon_{333} = 7560 \text{ M}^{-1} \text{ cm}^{-1}\)) 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 Eu\(^{3+}\) ion. The \(5\)D\(_0\) to \(7\)F\(_J\) transitions of EuL1 (\(\lambda_{\text{ex}} = 325 \text{ nm}\)) were found at 578 (\(J = 0\)), 585–603 (\(J = 1\)), 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 \(5\)D\(_0\) to \(7\)F\(_2\) transitions of Eu\(^{3+}\) ions in water is 0.5% (Table S2†).

Fluorimetric titration studies of EuL1

With EuL1 in hand, its binding properties towards Cu\(^{2+}\) ions were investigated. Upon the addition of 1 equiv. of Cu\(^{2+}\) ions (CuCl\(_2\) as the source of Cu\(^{2+}\) 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 $K_B = 1.5 \times 10^4 \text{M}^{-1}$ (inset of Fig. 5a). The detection limit of EuL₁ towards H₂S was calculated according to the $3\delta/\text{slope}$ 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 EuL1–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, EuL1–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 EuL1–Cu$^{2+}$ towards H$_2$S.

Mechanistic studies

The binding mechanisms of EuL1 towards Cu$^{2+}$ ions and the EuL1–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 illustrates the ratio of $^5D_0 \rightarrow ^7F_J$ ($J = 0$ to 4) emission bands of EuL1, EuL1 + Cu$^{2+}$ and EuL1 + Cu$^{2+}$ + H$_2$S$^a$.

![Fig. 7](image-url)  
Top: proposed binding mechanism of EuL1 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).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>$^5D_0$</th>
<th>$^7F_0$</th>
<th>$^7F_1$</th>
<th>$^7F_2$</th>
<th>$^7F_3$</th>
<th>$^7F_4$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EuL1</td>
<td>0.01</td>
<td>1</td>
<td>1.22</td>
<td>0.08</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>EuL1 + Cu$^{2+}$</td>
<td>0.08</td>
<td>1</td>
<td>1.86</td>
<td>0.15</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>EuL1 + Cu$^{2+}$ + H$_2$S</td>
<td>0.48</td>
<td>1</td>
<td>3.98</td>
<td>0.15</td>
<td>1.95</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ All spectra were acquired in water with excitation at 325 nm.
aza-18-crown-6–Cu$^{2+}$ complexes, causing significant luminescence quenching. Moreover, the binding of Cu$^{2+}$ would also provide a favourable conformation for forming a new 1 : 1 complex with H$_2$S. Upon the addition of H$_2$S, the emission profile of EuL1 changed significantly, $\Delta$F = 2/3$\Delta$F = 1 for [EuL1 + Cu$^{2+}$ + H$_2$S], and the intensity ratio was about >200% higher for [EuL1] and [EuL1 + Cu$^{2+}$]. 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 Cu$^{2+}$ ion via CuS precipitation. This proposal is further supported by the HRMS spectrum of the EuL1–Na$_2$S complex (Fig. S7†) and the $^1$H NMR signals of LaL1 were sharpened. These results suggested new complex formation after the displacement of the Cu$^{2+}$ ion via CuS precipitation.

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 Cu$^{2+}$ ions (Fig. 9a). This result indicated that the carbonyl linker of aza-18-crown-6 may be too rigid for coordination between Cu$^{2+}$ and pyridine, which could be essential for Eu emission quenching. Without the aza-crown moiety, EuL3 also showed no luminescence quenching towards Cu$^{2+}$ (Fig. 9b), suggesting DO3A–Eu$^{3+}$ is stable with Cu$^{2+}$ and the aza-crown motif is important for the Cu$^{2+}$ 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 Cu$^{2+}$ ions (Fig. 9c and d). These results indicated that the pyridine moiety of the chromophore is essential for the binding of Cu$^{2+}$ 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(Eu$^{3+}$)–pyridine–aza-crown motif, and studied its consecutive binding properties towards Cu$^{2+}$ and H$_2$S extensively. EuL1 binds to Cu$^{2+}$ ions selectively ($K_B = 1.2 \times 10^7$ M$^{-1}$) inducing 17-fold luminescence quenching and forming a 1 : 1 stoichiometric complex (EuL1–Cu$^{2+}$), which responds to H$_2$S 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–Na$_2$S) with $K_B = 1.5 \times 10^4$ M$^{-1}$. Without Cu$^{2+}$ ions, EuL1 shows non-specific binding towards H$_2$S with only a 5-fold luminescence enhancement. These results indicate that the Cu$^{2+}$ ion may pre-organize the conformation of EuL1 and facilitate the formation of the EuL1–Na$_2$S complex. The studies
on this unprecedented Cu²⁺-assisted luminescence enhancement of Eu emission are still ongoing. With long-lived Eu emission, reversible binding properties, an instantaneous response and high selectivity towards H₂S, this Eu-based luminescence “off-on” gate could find suitable applications for H₂S imaging in biological systems.

Acknowledgements

This work is funded by the Peking University Shenzhen Graduate School (Key State Laboratory of Chemical Genomics open-project fellowship program), grants from Shenzhen Science and Technology Innovation Committee (GRG2/14-15/013013), Hong Kong Polytechnic University central Research Grant (G-UC08), Natural Science Foundation of China (21471015 and 203012), Hong Kong Polytechnic University Research Grant (G-UC08), Natural Science Foundation of China (21401158) and HKBU and HKPolyU Joint Research Programme (RC-ICRS/15-16/02F-WKL02F-WKL).

Notes and references


The preparation and characterization of LaLa1 are available in the ESL.


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