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ARTICLE

A Highly Selective On-Off-On Responsive Lanthanide (III) based Probe for Recognition of Copper and Hydrogen Sulfide †

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The development of europium (III) based probe (**EuL1**) for detection of Cu (II) ion and hydrogen sulphide was presented. With addition of Cu (II) ion, **EuL1** displayed a greatest quenching among other cations. The binding constant was $74026 \pm 2899 M^{-1}$. Once combined with Cu (II) ion, **EuL1Cu** demonstrated high specificity for hydrogen sulfide among other organic and inorganic sulfur compounds. **EuL1Cu** exhibited on-off-on type luminescence change with alternately addition of Cu (II) ion and H_2S along with reversible forming-separating of the complex.

Introduction

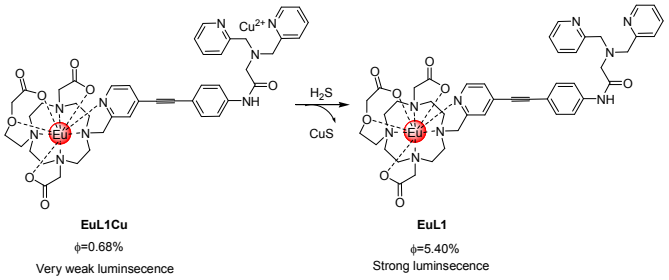
Hydrogen sulfide (H_2S) emerges as an important gaseous signaling molecule and plays an important role in various physiological processes, such as reduction of blood pressure,[1] relaxation of vascular smooth muscles,[2] inhibition of insulin signaling,[3] and regulation of inflammation.[4] Moreover, concentration of H_2S is changed in some diseases, including Down's syndrome,[5] Alzheimer's disease.[6] Therefore, the development of sensitive and selective methods for recognition of sulfide ions is a very crucial way for understanding physiological and pathological function of H_2S in detail.

Classical techniques, including methylene blue method[7] and the sulfide ion-selective electrode method,[8] have been commonly applied in detection of H_2S. However, there are some disadvantages since they are destructive method and require homogeneous sample. Therefore, development of fluorescent probe is a good way to detect H_2S due to a simple, sensitive, selective and non-invasive detection method. To date, there are several H_2S probes which had been developed.[9] Among these probes, there are three strategies for detection of H_2S, such as reduction of azides to amines,[10-13] nucleophilic reaction[14-15] and copper sulfide precipitation.[16-18] However, the first two were irreversible and time-consuming.[19-21] Therefore, copper sulfide precipitation had drawn attention and is relies on the specific reaction of H_2S to Cu (II) ion to give out fast, stable and low-solublility product CuS ($K_{sp}=6.3x10^{-36}$).[22]

To date, there are few organic based H_2S probes which had been developed.[9a-e] Ngano and co-worker use this method for detection of H_2S in living cells.[16] However, the drawback of organic dye based probes is a susceptibility to photobleaching, due to their small Stokes shifts. Thus, development of lanthanide (III) complexes, in particular of europium (III) complexes, are frequently utilized in biological applications such as responsive probes due to their large stoke shift, long lived time for the elimination of biological auto-fluorescence.[23a-d]

To our best knowledge, the first lanthanide (III) probe for recognition of H_2S was developed by Faulkner's group which relied on reduction of azides to amines to generate bright amine species with a six-fold enhancement after just 5 minutes.[24] However, there are some disadvantages of this probe include poor quantum yield (0.54%) and required time-gated method for detection of H_2S.

In this work, a new water-soluble europium (III) complex with use of dipicolylamine as a receptor was synthesized without water molecule directly coordinating europium (III) ion. Thus, no non-radiative process was happened in the system. As shown in **Scheme 1**, its sensitisation mechanism relies on copper sulfide precipitation. The luminescence of **EuL1** was almost completely quenched by binding of Cu (II) ion to form a dipicolylamine complex moiety. With the addition of H_2S (NaHS was used as H_2S source in this article), the removal of Cu (II) ion resulted in a recovery of the luminescence of **EuL1**. There was 8 fold of enhancement in terms of quantum yield. Among the inorganic and organic sulphur compounds, it is selective towards H_2S and this selective response allows **EuL1Cu** to be a very useful sensor for recognition of H_2S.



Scheme 1. Reaction mechanism for luminescence response of **EuL1Cu** towards H_2S.

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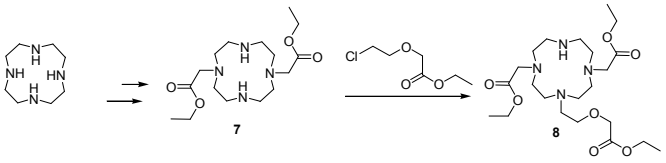
Results and discussion

Synthesis and characterization of the EuL1

Synthetic routes to **EuL1** are depicted in **Scheme 3.** Its eight steps synthetic routes of **EuL1** can be divided into four main parts, including synthesis of the chromophore, then connection between compound **8** and the chromophore, then ligation with the receptor DPA and then deprotection and complexation. Reaction of 1.1 eqv of $(Boc)_2$ and 4-ethynylaniline yielded the protected compound **1** in 62% yield. This protection was vital since Boc-protection could decrease the polarity of the product to allow for easy purification and prevent the reaction of the amine in the following reaction. Compound **2** was prepared by the Sonogashira coupling between compound **1** and (4-bromopyridin-2-yl)methanol in presence of trace amount of $PdCl_2(PPh_3)_2$ catalysis to give the product in 46% yield. Mild apple reaction was used to convert alcohol into bromine to yield compound **3** (yield 48%).

Before continuing to next step, the synthesis of compound **8** was protected with ethyl ester protection group showed in **Scheme 2**. The synthesis of compound **7** followed patent WO 2011049961. Side arm ethyl 2-(2-chloroethoxy)acetate was simply mixing with 2-chloroethanol and ethyl 2-bromoacetate in presence of NaH with yield 42%. Then, side arm ethyl 2-(2-chloroethoxy)acetate was refluxed with compound **7** for 2 days with yield 56%. Then, compound **8** was reacted with bromine derivative **3**. (yield: 37%) Compound **4** was deprotected with the use of TFA. (yield:80%) Then, compound **5** was first reacted with chloroacetyl chloride in the presence of TEA.

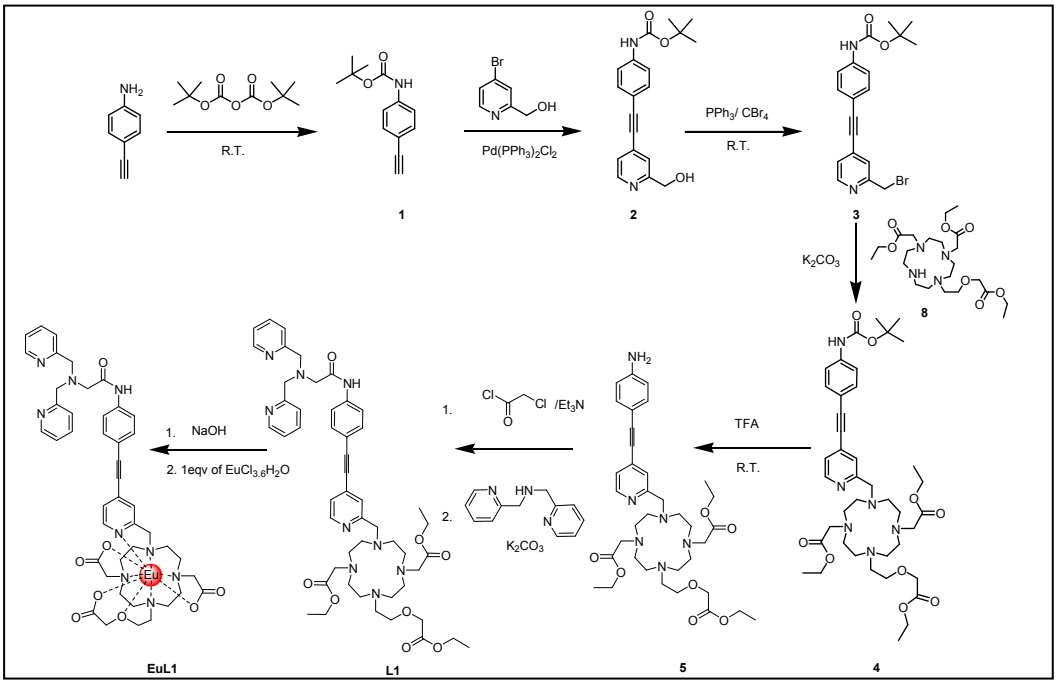
Without further purification, it reacted with bis(pyridin-2-ylmethyl)amine (DPA) with the final product yield 35%. **L1** was deprotected with NaOH and was purified by recrystalization in mixture of diethyl ether and methanol. The deprotected **L1** was refluxed with 1.0 eqv of $EuCl_3.6H_2O$ to yield **EuL1**. The product was characterized by high-resolution mass spectra (ESI+) which peaks corresponding to the double protonated **EuL1** complexes and isotopic pattern was observed.



Scheme 2. Synthetic route of the **8**.

Photophysical properties of EuL1 & EuL1Cu.

The UV-vis spectra of **EuL1** and **EuL1Cu** are presented in **Figure 1. EuL1** and **EuL1Cu** complexes showed similar absorption bands with the peak maxima centred at 325 nm which corresponds to π to π* transitions of the aromatic chromophore moieties. Their molar extinction coefficients were 11994 and 11374 M^{-1} respectively. The excitation spectra were very similar to the corresponding absorption spectra, indicating there was energy transfer from the chromophore moieties to the europium (III) metal centres. Upon an increase in concentration of Cu (II) ion (0-2 eqv Cu (II) ion) and on the excitation at 350 nm, it displayed narrow structured emission patterns of $^5D_0 \rightarrow {}^7F_J$ (J=0-4) transitions characteristic of Eu (III) ions. (**Figure 2**) The luminescence of **EuL1** was quenched upon the formation of dipicolylamine-Cu (II) complex moiety. During titration, the presence of one sharp component centred at 580 nm corresponding to the $^5D_0 \rightarrow {}^7F_0$ transition of Eu (III) complexes, which was indicative of a single species in solution.



Scheme 3. Synthetic route of the **EuL1**.

The magnetic dipole transition $^5D_0 \rightarrow {}^7F_1$ (I_{MD}) is independent of the crystallographic site of the Eu (III) ions and the electric dipole transition $^5D_0 \rightarrow {}^7F_2$ is hypensensitive (I_{ED}) towards the Eu (III) ions. Their corresponding ratios (I_{ED} /I_{MD}), which usually gives information of the symmetries and chemical environments of Eu (III)

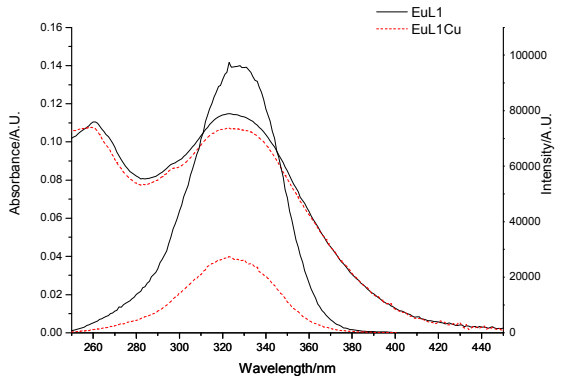


Figure 1. UV/Vis absorption spectra (solid line), excitation spectra (dotted lines, Eu, λ_{em} =616 nm) (blue line: **EuL1** red line: **EuL1Cu** (0.01M HEPES, pH 7.4).

ions, were 3.70 (**EuL1**) and 3.78 (**EuL1Cu**), indicating that symmetries of the Eu (III) ions were not be affected by titration of Cu (II) ion. (**Figure 2**) According to job's plot (Figure S4, Supporting Information), there was formation of 1:1 binding mode between **EuL1** and Cu (II) ion and ESI-Ms (Figure S2, Supporting Information) also showed similar results. Moreover, the binding constant (K) derived from fluorescence titration data was $74026 \pm 2899 M^{-1}$. (Figure S6, Supporting Information) It was larger than binding constants towards other cations inculding Fe(II)ion ($7951 \pm 936 M^{-1}$), Co(II) ion ($16713 \pm 3342 M^{-1}$) and Ni(II) ion ($15210 \pm 3042 M^{-1}$).(Figure S7-S9, Supporting Information) Detection limit for Cu (II) ion was $9.6 \pm 0.1 \mu M$. (Figure S5, Supporting Information)

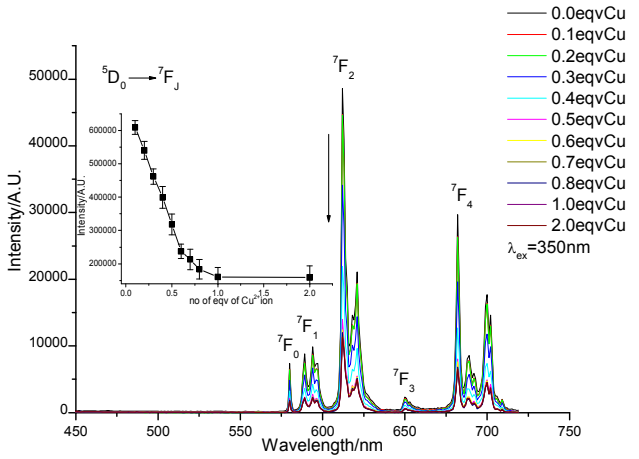


Figure 2. Emission spectra of 10 μM aqueous solution upon addition of aliquots of various equiv of Cu (II) ions with respect to Eu**L1** (0.01M HEPES, pH=7.4, λ_{ex}=350nm).

The average hydration states of **EuL1 and EuL1Cu** were determined by the measurement of luminescence lifetimes in H_2O and D_2O upon emission at 612 nm. The lifetime of Eu (III) complexes in D_2O was 1.3 ms which was longer than that in H_2O (0.97ms). They had similar lifetime in H_2O and HEPES. Based on their lifetimes in H_2O and D_2O, q value was calculated as zero in absence or presence of Cu (II) ion, indicating no water molecule directly coordinating with Eu (III) ion. The quantum yields were determined using quinine sulfate (0.1 M sulfuric acid, (Φ=0.577)). The relative quantum yields were 5.40% (**EuL1**) and 0.68% (**EuL1Cu**).

Selectivity of EuL1 towards cations

Its luminescent response of **EuL1** was also measured in 10 mM HEPES buffer (pH 7.4) upon the addition of various cations. (**Figure 3**.) The addition of large amount of the important biological cations such as 0.1M Na (I) ion, 0.1M K (I) ion, 0.1mM Ca (II) ion and 0.1mM Mg (II) ion produced negligible change in the emission of **EuL1**. Except for Cu(II), Co (II) and Ni (II) ion (slightly quenched for Co (II) and Ni (II) ion) , other cations e.g. Zn (II) ion, Hg (II) ion and Cd (II), Ba (II) ion, Mn (II) ion, Fe (II) ion, displayed no significant quenching signal of **EuL1**. Addition of Cu (II) ion can quench **EuL1** owing to coordination of dipicolylamine to paramagnetic Cu(II) ion centre. Therefore, the highest quenching efficiency (I_0-I)/I_0x100%=76%) was resulted. Moreover, **EuL1** can distinguish Cu (II) ion from Cu (I) ion and its luminescent response was not affected by anion such as ATP, GSH, cysteine, BSA…etc. In Figure S11, Supporting Information, **EuL1Cu** was not affect by the presence of various cations, including Na(I) ion, K(I) ion, Ca(II) ion, Mg(II) ion, Hg(II) ion, Cd(II) ion, Mn(II) ion and Fe(II) ion. While NaHS was added into the solution of **EuL1Cu** and heavy cations, the emission was recovered. In this case, the sensing of H_2S could not be affected by the presence of common metal cations.

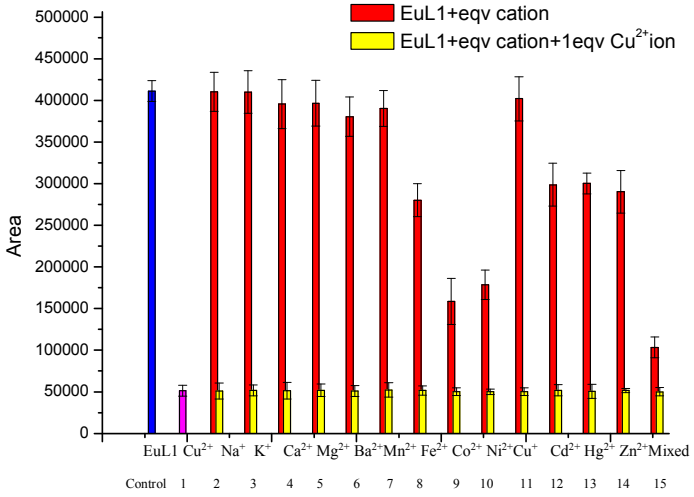


Figure 3. The luminescence intensity changes of [**EuL1**] (10 μM) in 10 mM HEPES with/without Cu(II) ion (excitation: 350 nm). Control 1: **EuL1 only**, 1: **EuL1**+1eqv Cu(II)ion only, 2: 0.1MNa(I) ion , 3: 0.1MK (I) ion,4: 0.1mM Ca (II) ion,5: 0.1mM Mg (II) ion,6: 1eqv Ba(II) ion,7. 1eqv Mn (II) ion, 8. 1eqv Fe(II) ion, 9. 1eqv Co (II) ion, 10. 1eqv Ni (II) ion, 11: 1eqv Cu (I) ion, 12: 1eqv Cd (II) ion, 13: 1eqv Hg (II) ion, 14: 1eqv Zn (II) ion, 15 (red bar): mixture of cations from 2 to 14, 15 (yellow bar): mixture of cations from 2 to 14 +1eqv Cu(II)ion

Measurement of triplet of Gd (III) complexes

To elucidate the energy transfer process of **EuL1**, the energy levels of the triplet should be estimated. The singlet and triplet energy levels were estimated by referring to UV−vis upper absorption edge of the Gd(III) complexes and phosphorescence spectra. The singlet

($^1\pi\pi^*$) energy level of **L1** was determined to be approximately 410 nm (24390 cm^{-1}). The triplet energy level of **L1** was not affected significantly by the lanthanide ion, and the lowest lying excited level of Gd(III) ion was located at 32150 cm^{-1}. Based on this, the phosphorescence spectra showed a red shift (peak at 507nm (19723cm^{-1})). According to experimental results, the schematic energy level diagram showing the energy transfer process is depicted in **Figure 4**. The triplet energy levels of **L1** is higher than $^5D_0$ level (17200cm^{-1}) of Eu^{3+} ion and their energy gaps between triplet and $^5D_0$ level more than 2000cm^{-1}. According to Latva's empirical rule, energy gaps > 2500cm^{-1} is optimal ligand to metal transfer process for Eu (III) ion.[25]

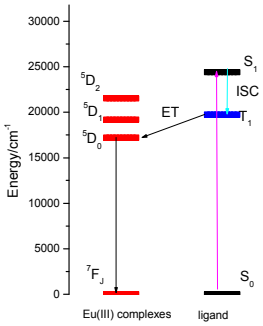


Figure 4. Schematic energy level diagram and energy transfer process of **EuL1**.

Luminescence response of EuL1Cu and S^{2-}.

Due to the strong affinity of H_2S with Cu (II) ion, the luminescence of **EuL1Cu** could be recovered gradually during titration with H_2S. (**Figure 5**.) It showed 8 folds enhancement in terms of quantum yield between the quench and unquenched complex. During titration, their shapes of hypersensitive splitting of $^5D_0 \rightarrow {}^7F_2$ were very similar, suggesting the metal ions experienced similar chemical environment. The detection limit was 2.7 ± 0.1μM. (Figure S10, Supporting Information) We further performed several control experiments to show the selectivity for H_2S, such as GSH, cyteine. But no enhancement was observed. Moreover, if titration only involved **EuL1** and NaHS (even in excess amount of NaHS), the response of **EuL1** had showed no significant change. GSH and cyteine had similar case.

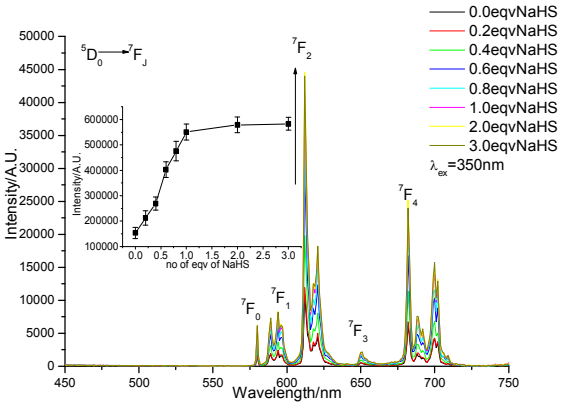


Figure 5. Emission spectra of 10 μM aqueous solution upon addition of aliquots of various equiv of NaHS with respect to **EuL1Cu** (0.01M HEPES, pH=7.4, λ_{ex}=350nm) .

Selective Optical Response of EuL1Cu towards Various Anions

The evaluation of the response of **EuL1Cu** to various anions was done and its luminescent response of **EuL1Cu** was also measured in 10 mM HEPES buffer (pH 7.4) upon the addition of various anions species, including sulfur species such as $NaHSO_3$, Na_2SO_4, $Na_2S_2O_3$, glutathione (GSH), cysteine and other important biological anions such as Na_2HPO_4, $NaHCO_3$, sodium citrate, sodium ascorbate, KI, KBr, pyrophospate, potassium acetate. (**Figure 6**.) The addition of ten eqv of sulfur species such as $NaHSO_3$, Na_2SO_4, $Na_2S_2O_3$, glutathione (GSH), cysteine produced negligible change for the emission of **EuL1Cu**. Since Cu (II) ion formed a very stable complex with dipicolylamine, especially for GSH and cysteine which had SH group but did not give any interference of the signal of **EuL1Cu**. The addition of NaHS to the solution of **EuL1Cu** and sulfur species leaded to recovery of luminescence, which indicated that the H_2S was the only one to form precipitation of copper sulfide (CuS) and regenerated **EuL1**. For other important biological anions such as Na_2HPO_4, $NaHCO_3$, sodium citrate, sodium ascorbate, KI, KBr, pyrophospate, potassium acetate, there were no observable responsive change of **EuL1Cu** due to high stability of Cu(II) ion-dipicolylamine complex. In Figure S12, Supporting Information, the reverse titration which NaHS was first added to **EuL1Cu** to regenerate **EuL1**, then other anions was added then, revealed that their luminescence was similar with the luminescence of **EuL1Cu** with NaHS. **EuL1** did not interfere with the other anions.

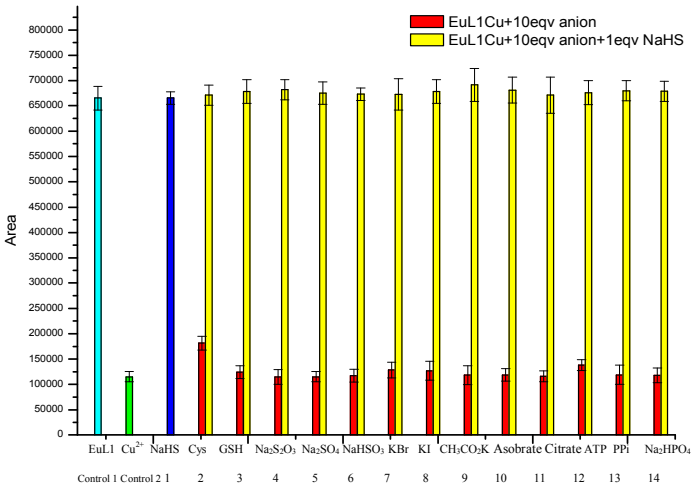


Figure 6. The luminescence intensity changes of [**EuL1Cu**] (10 μM) in 10 mM HEPES with/without (excitation: 350 nm). Control 1:**EuL1**only, Control 2: **EuL1** + 1eqv Cu(II) ion 1. **EuL1** + 1eqv Cu(II) ion+1 eqv NaHS, 2: cysteine (Cys), 3: glutathione (GSH), 4: $Na_2S_2O_3$,5: Na_2SO_4, 6: $NaHSO_3$,7: KBr, 8: KI, 9: potassium acetate, 10: sodium ascorbate, 11: sodium citrate, 12:ATP, 13: pyrophosphate(PPi),14: Na_2HPO_4.

On-Off response of EuL1Cu and S^{2-}

On-off switchable change in the luminescence of the complex can be observed by alternative addition of Cu (II) ion and H_2S to solution of **EuL1**. It has been shown that such reversible interconversion can be repeated in four cycles by alterative addition of Cu (II) ion and H_2S, suggesting that **EuL1** can be a good candidate for development of on-off-on probe for Cu (II) ion and H_2S. (**Figure 7.**)

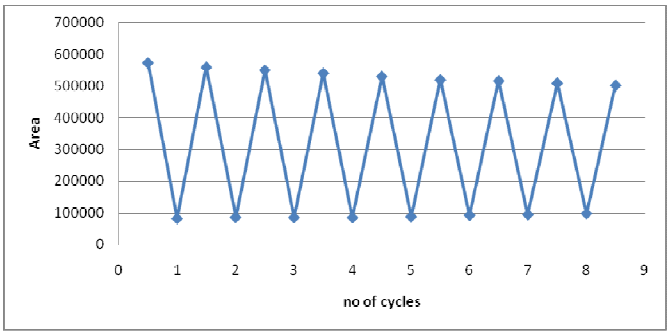


Figure 7. Luminescence intensity of **EuL1** (10uM) in 10mM HEPES (pH 7.4) on alternate addition of Cu (II)-NaHS. Each measurement was done after 60 min equilibrium from addition of each Cu (II) ion /NaHS.

Conclusions

Detailed photophysical solution studies have been performed. **EuL1** demonstrated a high selectivity and sensitivity for Cu (II) ion. The binding constant was 74026±2899M^{-1} and corresponding detection limit was 9.6±0.1μM. Upon **EuL1** binding with Cu (II) ion, it becomes a selective responsive H_2S probe. The detection limit was 2.7±0.1μM. The reversible binding between **EuL1** and Cu (II) ion or **EuL1Cu** and H_2S showed the on-off-on type luminescence which can allow this probe to be used as a monitoring system.

Experimental

General Methods.

Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Acetonitrile (ACN) and dichloromethane (DCM) were distilled from calcium hydride. NMR spectra were recorded with a Bruker Ultrashield 400 Plus NMR spectrometer. All reactions were monitored using thin-layer chromatography (TLC) on Merck silica gel plates (Merck, Kieselgel 60, 0.25 mm thickness) with F_{254} indicator. ^{1}H NMR chemical shifts were referenced to internal $CDCl_3$ and then re-referenced to TMS (δ = 0.00 ppm). Mass spectra, reported as m/z, were obtained with The Micromass® Q-ToF 2 mass spectrometer.

Synthesis of **1**.

4-Ethylaniline (936mg, 0.8 mmol), $(Boc)_2O$ (2.18g, 1 mmol) were stirred in THF (10ml).The reaction mixture was filtered and purified by silica column chromatography eluting with PE:EA (15:1) to afford **1**. (yield:62%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (m, 9H, OCH_3), 3.01 (s, 1H, CH), 6.51 (s, br, 1H, NH) , 7.32 (m, J=8.6, 1H, ArH), 7.41-7.43 (m, 2H, ArH), ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ28.3, 80.9, 83.5, 116.2, 117.9, 132.9, 138.9, 152.3.MS (ESI). Calcd for $C_{13}H_{15}NO_2Na$ [$(M + 22)^+$] m/z 240.10. Found: m/z 240.13.

Synthesis of **2**.

1 (1.29g, 0.40 mmol) was dissolved in dry triethylamine (8 mL) and dry THF (4 mL) under an atmosphere of nitrogen. Copper(I) iodide (9.8mg, 0.005 mmol), triphenylphosphine (52mg, 0.02 mmol) and dichlorobis(triphenylphosphine)palladium(II) (35mg, 0.005 mmol) were added to the stirred solution. (4-bromopyridin-2-yl)methanol (752mg, 0.40 mmol) was added in and the mixture was heated to 70° C for 12 h. After cooling, the formed precipitate of triethylamine hydroiodide was filtered off and washed with THF. The combined filtrates were evaporated under reduced pressure, and the crude product was purified by silica column chromatography eluting with PE:EA (10:1) to afford **2**. (yield: 46%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H, CH_3), δ 4.76 (s, 2H, CH_2), 6.77 (s, 1H, NH), 7.27-7.48 (m, 6H, ArH), 8.51 (d, J=4.5, 1H, ArH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ29.7, 65.4, 82.5, 87.5, 95.7, 117.5, 119.5, 123.7, 125.5, 133.9, 134.3, 140.9, 0149.8, 153.8, 160.7. MS (ESI). Calcd for $C_{19}H_{20}N_2O_3$ [$(M + 1)^+$] m/z 325.15. Found: m/z 325.07.

Synthesis of **3**.

2 (113mg, 0.35mmol), PPh_3 (99.6mg, 0.38mmol) were mixed together and CBr_4 (132mg, 0.4mmol) was added to the reaction mixture and was allowed to stirred for three hours. The combined filtrates were evaporated under reduced pressure, and the crude product was purified by silica column chromatography eluting with PE:EA (20:1) to afford **3**. (yield: 37%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.52 (s, 9H, CH_3), δ 4.53 (s, 2H, CH_2), 7.09 (s, 1H, NH), 7.27 (d, J= 5.4Hz, 1H, ArH), 7.41-7.51 (m, 5H, ArH), 8.53 (d, J=5.1, 1H, ArH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ28.3, 33.3, 80.9, 85.7, 94.9, 115.8, 118.1, 124.6, 125.2, 132.9, 132.9, 139.7, 149.46, 152.4, 156.8. MS (ESI). Calcd for $C_{19}H_{19}BrN_2O_2$ [$(M + 1)^+$] m/z 387.06. Found: m/z 387.13.

Synthesis of **4**.

3 (108mg, 0.28 mmol), **8** (133mg, 0.28 mmol) and K_2CO_3 (77mg, 0.56mmol) were stirred in ACN (15ml) under reflux overnight .The reaction mixture was filtered and purified by silica column chromatography eluting with DCM:MeOH (20:1) to afford **4**. (yield:37%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23-1.29 (m, 9H, CH_3), 1.52 (s, 9H, OCH_3), 2.40-2.98 (m, br, 22H, CH_2), 3.25-3.81 (m, 4H, CH_2), 4.12-4.18 (m, 8H, CH_2), 7.23 (d, J= 4.9Hz, 1H, ArH), 7.28 (s, 1H, ArH), 7.44-7.47 (m, 3H, ArH), 7.56-7.58 (m, 2H, ArH), 8.28 (d, J=5.04, 1H, ArH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ14.1, 28.3, 49.6, 50.3, 52.0, 55.9, 58.90, 60.9, 61.2, 67.6, 68.4, 80.6, 85.4, 95.5, 115.2, 118.3, 123.9, 125.4, 132.7, 132.9, 140.2, 149.1, 152.6, 158.3, 170.2, 172.9. MS (ESI). Calcd for $C_{41}H_{60}N_6O_9$ [(M + 1)+] m/z 781.44. Found: m/z 781.33.

Synthesis of **5**.

4 (68mg, 0.1 mmol) and TFA (1ml) were stirred in DCM (2ml) overnight. The reaction mixture was filtered and washed by DCM:diethyl ether (1:1) to afford **5**. (yield:80%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18-1.25 (m, 9H, CH_3), 2.15-2.90 (s, 22H, CH_2), 3.20-3.50 (m, 4H, CH_2), 4.07-4.18 (m, 8H, CH_2), 4.51 (s, br, 2H, NH_2),7.14 (ms, 1H, NH), 7.23-7.29 (m, 2H, ArH), 7.45-7.53 (m, 5H, ArH), 8.28 (d, 1H, ArH)ppm; ^{13}C NMR (100 MHz, $CDCl_3$) 14.1, 49.6, 50.3, 51.2, 52.8, 56.0,57.1, 60.7, 60.8, 61.2, 67.9, 80.74, 86.4, 95.9,114.6, 124.2, 125.2, 125.9, 133.4, 149.0, 158.1, 170.7. MS (ESI). Calcd for $C_{36}H_{52}N_6O_7$ [$(M + 1)^+$] m/z 681.39. Found: m/z 681.43.

Synthesis of **8**.

7 (400mg, 1.16 mmol), ethyl 2-(2-chloroethoxy)acetate (192mg, 1.16mmol) and K_2CO_3 (160mg, 1.16mmol) were stirred in ACN (12ml) under reflux 2 days .The reaction mixture was filtered and purified by silica column chromatography eluting with DCM:MeOH (20:1) to afford **8**. (yield:56%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26-1.31(m, 9H, CH_3), 2.70 (s, 4H, CH_2), 2.83-2.84 (s, 6H, CH_2), 3.12-3.21 (m, 7H, CH_2), 3.55 (s, 4H, CH_2), 3.68-3.71 (m, 3H, CH_2), 4.10-4.25 (m, 8H, CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ14.1, 14.2, 42.6, 44.8, 49.1, 49.9, 53.3, 4.7, 56.0, 60.5, 60.8, 61.1, 67.9, 68.4, 68.0, 169.9, 171.1. MS (ESI). Calcd for $C_{22}H_{42}N_4O_7$ [(M + 1)+] m/z 475.31. Found: m/z 475.59.

Synthesis of **L1**.

5 (50mg, 0.073mmol), triethylamine (0.02ml) were stirred in DCM (10ml) and chloroacetylchloride (0.006ml, 0.073mmol) was added into the solution mixture and were allowed to react a half day. The reaction mixture was filtered and combined filtrates were evaporated under reduced pressure. Without further purification, the reaction mixture were reacted with dipicolylamine (0.018ml, 0.1mmol) and K_2CO_3 (20.7mg, 0.15mmo) were refluxed in ACN (6ml) for three days. The reaction mixture was filtered and combined filtrates were evaporated under reduced pressure. The reaction mixture was then washed by water several times and purified by silica column chromatography eluting with gradient from DCM to DCM:MeOH (3:1) to afford **4**. (yield:35%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26-1.30 (m, 9H, CH_3), 2.18-2.85 (m, br, 26H, CH_2), 3.77 (s, br,7H, CH_2), 4.14-4.20 (m, br, 7H, CH_2), 7.22-7.24 (m, 4H, ArH), 7.36-7.38 (m, 4H, ArH),7.61-7.68 (m, 4H, ArH), 8.35 (s, 1H, NH)ppm, 8.67-8.12 (m, 3H, ArH); ^{13}C NMR (100 MHz, $CDCl_3$) 14.3, 49.6, 50.3, 52.4, 55.9, 59.0, 60.2, 61.0, 65.4, 66.8, 67.6 68.5 69.31, 80.6, 85.6 95.5 114.3 118.4 120.9, 123.4, 124.2, 125.4 131.6, 132.7 136.2, 141.7 147.4 149.2 152.6, 155.3 158.4, 170.2 172.9. Elemental analysis calcd (%) for $C_{50}H_{65}N_9O_8$: C 65.27, H 7.13, N 13.71; found: C 65.91, H 7.85, N 14.38.MS (ESI).Calcd for $C_{50}H_{65}N_9O_8$ [(M + 1)$^+$] m/z 920.50. Found: m/z 920.53.

Synthesis of **EuL1**.

L1 (10mg, 0.011mmol) was stirred in 0.05M NaOH (1ml) for a half day until the solution became clear. The reaction mixture was filtered and neutralized to pH 7 and re-crystallized in mixture of diethyl ether and methanol several time to yield deprotected **L1** which was reflux with 1 eqv. of europium(III) chloride hexahydrate (4mg, 0.011mmol) in water overnight. The reaction mixture was tuned pH to 6 and filtered and combined filtrates were evaporated under reduced pressure. The product was re-crystallized in chloroform to yield **EuL1**. (yield 41%) ^{1}H NMR (400 MHz, D_2O) δ 2.02-3.69 (m, 10H, CH_2), 4.23-4.33 (m, 10H, CH_2), 7.73-7.85 (m, 7H, ArH), 8.23 (s, 4H, ArH),8.30-8.57 (m, 3H, ArH), 9.44 (s, 1H, ArH) ppm, 8.67-8.12 (m, 3H, ArH). Elemental analysis calcd (%) for $C_{44}H_{52}EuN_9O_8$: C 53.55, H 5.31, N 12.77; found: C 53.89, H 5.61, N 13.08. Retention time (HPLC): = 7.35min. HRMS (+):492.6581 $(M+2H)^{2+}$ $[C_{44}H_{52}EuN_9O_8]^{2+}$requires 492.6563).The isotopic distribution matches closely with the simulated spectrum.

HPLC analysis.

The reverse-phase HPLC analysis of complex was carried out at room temperature by using VisionHT C18 Highload 250 x 4.6mm 5um column. The mobile phase was 0.05% trifluoroacetic acid in Milli-Q water and 0.05% trifluoroacetic acid (TFA) in MeCN solvent system, and the flow rate was 1.0 mL min^{-1}. The solvent gradient program is listed in below table.

Time(min)	0.05% TFA in Milli-Q water (%)	0.05% TFA in MeCN (%)
0	90	10
10	20	80
15	0	100

Spectroscopic Measurements.

UV-Visible absorption spectra of lanthanide complexes were recorded by a HP UV-8453 spectrophotometer and single-photon luminescence and lifetime spectra were recorded using a Edinburgh Instrument FLS920 Combined Fluorescence Lifetime and Steady state spectrophotometer that was equipped with a single photon counting photomultiplier in Peltier Cooled Housing (185 nm to 850 nm). The overall quantum yield of the sensitized europium (III) luminescence of the complex was measured at room temperature and was cited relative to a reference solution of quinine sulfate in 0.1 M H_2SO_4 (Φ_r = 57.7%). The overall luminescence quantum yield of the complexes was calculated according to eqn (1),

Where $$\Phi_x = \Phi_r \left(\frac{\text{gradient}_x}{\text{gradient}_r}\right)\left(\frac{n_x}{n_r}\right)^2$$ eqn (1)

n = refractive index of solution

The subscript r denotes the reference, and the subscript x implies an sample. The refractive index is assumed to be equivalent to that of the pure solvent: 1.33 for water at room temperature. All data reported are average of at least three independent measurements.

Metal Ion Selectivity Measurements.

The luminescence emission from **EuL1**(10 uM) was measured in 10 mM HEPES buffer (pH 7.4, λ_{ex}=350 nm), with addition of various amounts of metal ions (1.0 of metals). Metals were added as $BaCl_2$, $MnCl_2$, $FeCl_2$, $CoCl_2$, $NiCl_2$, tetrakis(acetonitrile)copper(I) hexafluorophosphate, $CdCl_2$, $ZnCl_2$, and NaCl (100 mM), KCl (100 mM), $CaCl_2$ (1 mM), and $MgCl_2$ (1 mM). Then, Identical solutions were prepared with the addition of $CuCl_2$ (1.0 equiv of $CuCl_2$) to solution of metal ions and **EuL1**.

Binding Constant.

The binding constant was calculated form the emission intensity-titration curve based on eqn 2. [18]

$$\frac{I_0}{I-I_0} = \left(\frac{1}{f}\right)\left(\frac{1}{K_s[M]} + 1\right) \qquad \text{eqn (2)}$$

Here, I_0 is the intensity of free **EuL1**, I is the intensity measured with addition of different amount of Cu (II) ion, f is the fraction of initial luminescence, [M] is the concentraton of Cu (II) ion and K_s is calculated as the ratio intercept/slope.

Detection Limit.

It was following the literature procedure and was calculated from a plot of the luminescence changes as a function of $\log[S^{2-}]$.[26] A linear regression curve was fitted to the intermediate values of the sigmoidal plot. The point at which this line crossed the ordinate axis was taken as the detection limit.

On-Off-On response experiment.

The luminescence emission from **EuL1**(10 uM) was measured in 10 mM HEPES buffer (pH 7.4, λ_{ex}=350 nm) on alternate addition of Cu (II)-NaHS with several concentration ratio (0:0, 10:0, 10:10, 20:10,20:20, 30:20, 30:30, 40:30, 40:40, 50:40, 50:50, 60:50, 60:60, 70:60, 70:70 uM, respectively). Each measurement was done after 60 min equilibrium from addition of each Cu (II) ion /NaHS.

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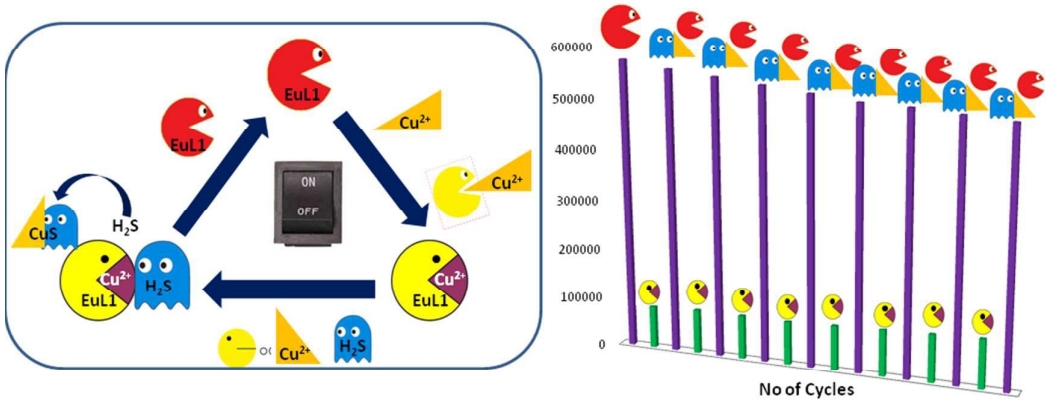
Graphical abstract



Europium (III) based probe (**EuL1**) for detection of Cu (II) ion and hydrogen sulphide has been designed which shows luminescent response to the two analytes. **EuL1** demonstrates a highly selective response towards Cu (II) ion among all other metal cations. The binding constant was found to be 74026 2899M^{-1} with a sensitive detection limit of 9.55 0.08μM. Once combined with Cu (II) ion, the stable **EuL1Cu** complex then shows specific binding response to hydrogen sulfide thus forming an on-off-on response when monitoring the luminescence change by alternate addition of Cu II and HS ions.