Reaction Based Europium Complex for Specific Detection of Cysteine over Homocysteine and Glutathione with Variable Temperature Kinetic Studies

Yuk-Wang Yip[a,b], Zhiyuan Yan[b], Ga-Lai Law[a,b]*, Wing-Tak Wong[a,b]*

Abstract: A water-soluble europium (III) based probe, **EuL,** has been designed and synthesised for selective recognition of cysteine (Cys) over other structurally similar thiols and amino derivatives due to a more stable and preferable seven membered ring formation. Addition of Cys shows a significant response with quenching of over 90 % of the initial signal (Hcys: 10.5 %，GSH: 3.6 % respectively). A good linear correlation between the emission intensities and concentrations of Cys has been established.

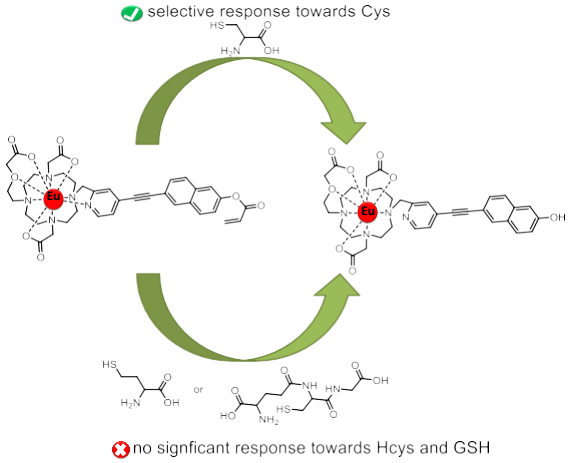
Introduction

Cysteine (Cys) plays a crucial role in various physiological processes, including redox homeostasis,[1] cellular growth[2] and glutathione (GSH) synthesis[3] as its concentration often signifies the presence of some specific diseases.[4] For instance, an elevated level of Cys is identified during the progression of Parkinson's disease[5] and associated with neurotoxicity[6] while decline levels of Cys is related to skin lesions, liver damage, edema, lethargy and hair depigmentation.[7] Therefore, recognition of Cys is of immense interest and significance.

Classical analytical methods for detection of Cys such as high-performance liquid chromatography (HPLC),[8] potentiometry[9] and capillary electrophoresis[10] have some drawbacks, including complicated and laborious pretreatment procedures and expensive instrumentation. Compared with other techniques, fluorimetry is an excellent and simple detection method due to the sensitivity, selectivity, convenience and low cost detection method. To date, various luminescent probes for recognition of Cys, including quantum dot based,[11] upconversion nanoparticle based,[12] organic dye based Cys probes[13] have been developed. However in these probes, the mechanism relies on cyclizaion reaction with aldehyde,[14] cleavage reaction by thiols,[15] Michael addition, [16a-h] thiolysis of sulfonate ester[17] and cleavage of selenium–nitrogen bonds by thiols.[18] The drawback is that only a few of these are water-soluble and enables discrimination of Cys from homocysteine (Hcys) and glutathione (GSH) due to the similarity in both structure and reactivity.

Moreover, most of them are organic based Cys probes which are susceptible to photobleaching. Therefore, the development of lanthanide (III) complexes, in particular those of europium (III) complexes, have drawn much interest due to their large stoke shift and long lived lifetimes which prevent photobleaching and allow for time gated techniques. [19]

In this work, a new water-soluble europium (III) complex **EuL** with an acrylate group as the reaction site[20] was designed and synthesized as shown in **Scheme 1** which also portrays its sensitisation mechanism. Among thiol derivatives such as Hcys, GSH, N-Acetyl Cysteine, H_2S and similar structural amino acids, our probe demonstrated a high selectivity towards Cys owing to a more kinetically favoured formation of a seven-membered ring from the acrylate group with Cys in an intramolecular cyclization. Its luminescent intensity was quenched significantly around 90 % in turns of quantum yield and demonstrated a good linear correlation between emission intensities and concentrations of Cys as well as a good stability in the physiological window (pH 4-8). To the best of our knowledge, this is the first lanthanide (III) based probe for selective recognition of Cys over Hcys and GSH



Scheme 1. Reaction mechanism for luminescent response of **EuL** towards Cys, Hcys and GSH.

Results and Discussion

Synthesis and characterization of the EuL

The synthetic routes of **EuL** require seven steps and are described in **Scheme 2.** This can be sub-divided into four main parts:- 1) the general synthesis of the chromophore, 2) incorporation of **5** into chromophore, 3) deprotection and metal complexation and finally 4) attachment of the acylate group. For compound **1**, this was prepared by Sonogashira coupling between (4-bromopyridin-2-yl)methanol and ethynyltrimethylsilane in the presence of trace amount of $PdCl_2(PPh_3)_2$ catalysis to give the product in 64 % yield[21]. Then, compound **2** was prepared by the deprotection of trimethylsiliane protecting group by Tetra-n-butylammonium fluoride (TBAF). Compound **1** was stirred with 1.5 eqv TBAF in THF for five hours with yield 82 %. Sonogashira coupling reaction between compound **2** and (6-bromonaphthalen-2-yloxy)(tert-butyl)dimethylsilane afforded compound **3**. (yield: 46 %) Then, compound **3** was stirred with 1.2 eqv methanesulfonyl chloride with 2.0 eqv of triethylamine

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at room temperature overnight to yield compound **4**. (yield: 48 %) The synthesis of compound **5** was followed by ref. 19a. Compound **6** was stirred with compound **5** with 2 eqv of K_2CO_3 overnight (yield: 52 %). Finally, deprotection of compound **6** by mixing with LiOH and then refluxed with 1.0 eqv of $EuCl_3.6H_2O$ formed compound **Eu7**. (yield: 56 %) This as the final step, compound **Eu7** was reacted with acryloyl chloride to yield **EuL** (yield: 48 %) The product was characterized by high-resolution mass spectrometry using positive electron spray ionisation (ESI+) with the main peak corresponding to the protonated **EuL** complex and confirmed by the Eu isotopic pattern.

Photophysical properties of EuL

The UV-vis spectra of **EuL** before and after addition of Cys are shown in **Figure 1** with no obvious difference, the peak maxima centred at 323 nm corresponds to the π to π* transitions associated to the aromatic chromophore moieties[22]. The molar extinction coefficients were found to be 1.44×10^3 and 1.51×10^3 M^{-1} respectively. The absorption spectra are very similar to the corresponding excitation spectra, and indicative of energy transfer occurring from the chromophore moieties to the europium (III) ion centre[23]. Upon addition of Cys (0-20 eqv Cys) under 350 nm excitation, it demonstrated quenching of the narrow structured emission patterns $^5D_0 \rightarrow {}^7F_J$ (J=0-4) transitions, which is characteristic of the Eu (III) ion (**Figure 2**). From the emission spectra it can be observed that there is one sharp peak at the $^5D_0 \rightarrow {}^7F_0$ transition centred at 580 nm, which is informative of a single species in solution. The ratio of magnetic dipole transition $^5D_0 \rightarrow {}^7F_1$ (I_{MD}) and the electric dipole transition $^5D_0 \rightarrow {}^7F_2$ (I_{ED}) generally provides information of the symmetries and chemical environments of the Eu (III) ion since I_{MD} is independent of the chemical site of the Eu (III) ions while I_{ED} is hypensensitive towards the Eu (III) ion. Upon calculation, the I_{ED}/I_{MD} ratio were 4.96 (without Cys) and 4.86 (with Cys), suggesting that symmetries of the Eu (III) ion were not affected by the presence of Cys. (**Figure 2**) The titration also shows a linear relationship between the luminescent intensity and concentration of Cys (5 μM to 80 μM). (**Figure 3**)

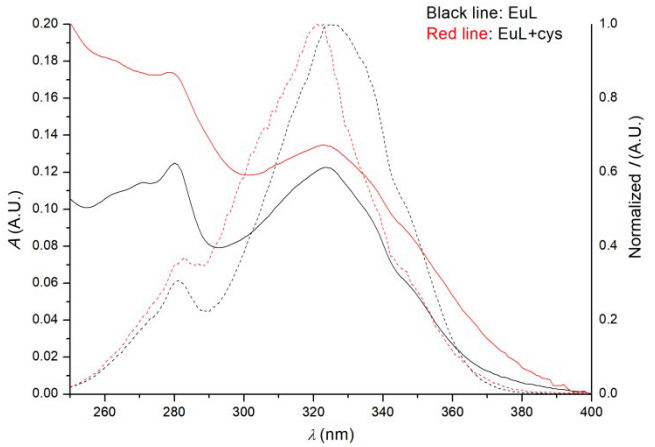


Figure 1. UV/Vis absorption spectra (solid line), excitation spectra (dotted lines, λ_{em} =612 nm) (Black line: **EuL** red line: **EuL**+10 eqv Cys (0.01M HEPES, pH 7.4). Measurement was done after 80min.

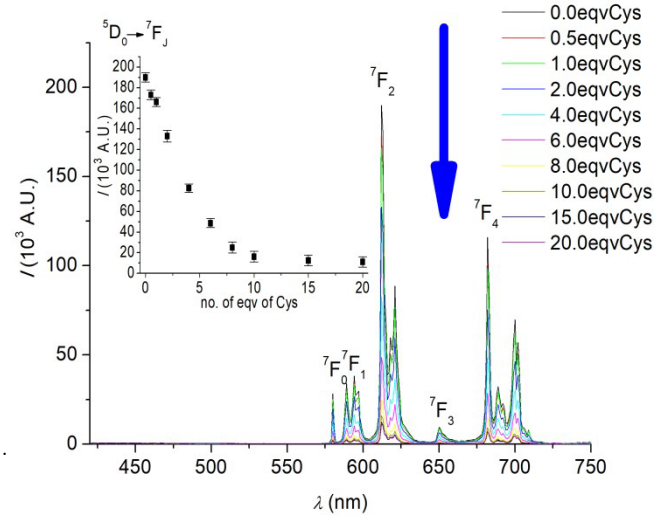
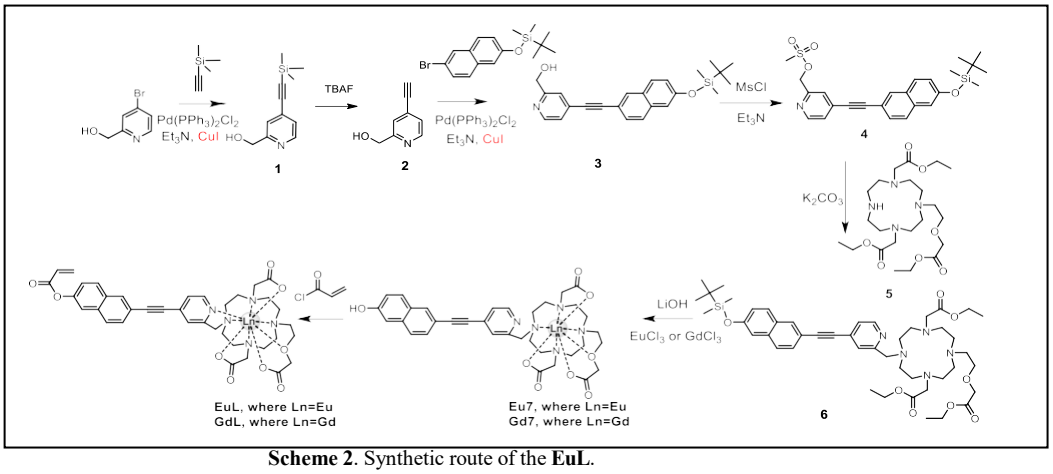


Figure 2. Emission spectra of 10 μM aqueous solution upon addition of aliquots of various equiv of Cys with respect to **EuL** (0.01 M HEPES, pH=7.4, λ_{ex}=350 nm). Insert: Luminescence response of europium emission intensity (λ= 612 nm) to changing Cys. Luminescence emission was measured after 80min.



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

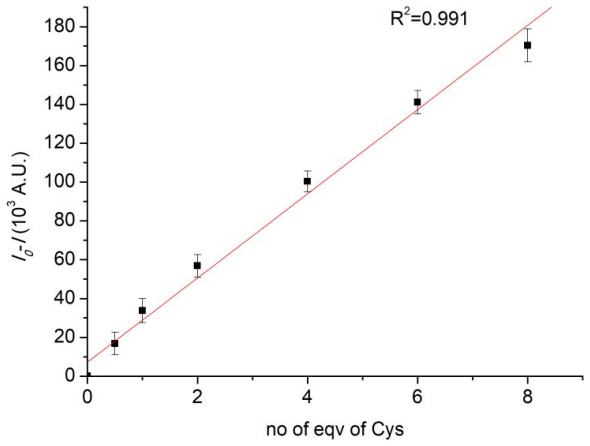


Figure 3. Linear luminescence response of 10 µM aqueous solution upon addition of aliquots of various eqv of Cys with respect to **EuL** (0.01M HEPES, pH=7.4, λ_{ex}=350 nm). Luminescence emission was measured after 80min.

Titrations with the Hcys and GSH have also been performed and are in the supporting information. (**Figure S10&S11**) Relative quantum yields were determined for all three analytes by using quinine sulfate (0.1 M sulfuric acid, (Φ=0.577)) a known standard.[24] The relative quantum yields (with 15% errors) are 6.43 (without Cys), and 0.710 (with Cys), 5.72 (with Hcys) and 6.20 (with GSH). In view of the quantum yields, it can be observed that the extent of quenching of luminescent intensity by Cys at around 89 % is much higher than that of Hcys (10.5 %) and GSH (3.6 %). To confirm that the mechanism is solely reaction based, we also looked at the average hydration states of **EuL** with and without 10eqv of analytes which confirms that for all three analytes there was no coordinating water molecule. The measurements with analytes were done after 80min. The hydration states are determined by the measurement of luminescence lifetimes in H_2O and D_2O upon emission at 612 nm.[25]. The lifetime of **EuL** without Cys in H_2O was 0.92 ms which was shorter than that in D_2O (1.24 ms) while the lifetime of **EuL** with Cys in H_2O was 0.87 ms, much shorter than that in D_2O (1.16 ms). The lifetimes were also measured in HEPES which corresponded to the values in H_2O. Based on the lifetimes in H_2O and D_2O, the q value was calculated as zero in the absence and presence of Cys, suggesting that there was no bound water molecule coordinating directly with Eu (III) ion and that the complex/geometric coordination was stable during the Cys titrations with no solvent or anion exchange.

Selectivity of EuL

The luminescent response of **EuL** towards thiol derivatives and amino acid was investigated in 10 mM HEPES buffer (pH 7.4) at room temp. (**Figure 4**) The addition of thiol derivatives such as N-Acetyl cysteine, cystine, CH_3SCH_3, $NaHSO_3$, H_2S, Na_2SO_4, $Na_2S_2O_3$ and amino acids such as histidine (his), valine (val), isoleucine (Iso), alanine (ala), arginine (arg), asparagines (asp), aspartic acid (asc), lysine (lys), methionine (met), serine (ser) produced no significant change in the luminescent intensity of **EuL**. It was worth noting that **EuL** could distinguish Cys from N-Acetyl cysteine and cystine. In the case of Hcys and GSH, the luminescent intensity was only slightly quenched. Addition of Cys to a mixture of **EuL** and other thiols or amino acids, it can be observed that the state of quenching is the same as that for a mixture of **EuL** and Cys, indicating **EuL** does have selective response towards Cys in the presence of other thiols or amino acids.. Reverse titration, in which Cys was first added to **EuL** followed by addition of other thiols or amino acids, was also performed for confirmation. Observations showed no significant effect caused by addition of other thiols or amino acids to a mixture of **EuL** and Cys, suggesting that the response of **EuL** towards Cys was also not altered by the presence of other thiols and amino acids.

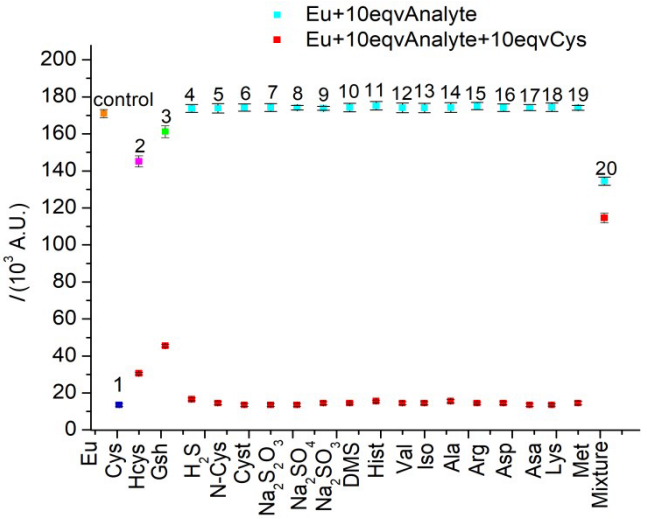


Figure 4. The luminescence intensity changes of [**EuL**] (10 µM) in 10 mM HEPES with/without analytes (excitation: 350 nm). Control: **EuL** only, 1: **EuL**+10 eqv Cys, 2: 10 eqv Hcys , 3: 10 eqv GSH,4: 10 eqv H_2S ,5: 10 eqv N-acetyl cysteine, 6: 10 eqv Cystine,7: 10 eqv $Na_2S_2O_3$, 8: 10 eqv Na_2SO_4, 9: 10 eqv Na_2SO_3, 10: 10 eqv CH_3SCH_3, 11: 10 eqv histidine, 12: 10 eqv valine, 13: 10 eqv isoleucine, 14: 10 eqv alanine, 15: 10 eqv arginine, 16: 10 eqv asparagines, 17: 10 eqv aspartic acid, 18: 10 eqv lysine, 19: 10 eqv methionine, 20: mixture of Hcys, GSH and H_2S, (cyan point): mixture of analytes from 1 to 19 (red point): mixture of analytes from 1 to 19 +10 eqv Cys. (For simplicity, the maximum intensities of I0-I of **EuL** with/without analytes are plotted

Sensing Properties of EuL

To further investigate the reactivity of **EuL** towards Cys, Hcys and GSH, time-dependent luminescent spectra were analyzed by monitoring the luminescent changes of the reaction mixture in HEPES (pH 7.4). On the addition of 10eqv of Cys into **EuL** at room temp, the emission intensity reached a plateau, maximising within 80 min while Hcys and GSH showed a smaller quenching of emission intensity with a much slower rate. (**Figure 5a**) Based on these results, **EuL** could detect Cys specifically over Hcys and GSH within 80 min. The faster reaction of **EuL** with Cys could be rationalised by the favourable cyclisation product and pKa values. Firstly, pKa of Cys at 8.30 is lower than that of Hcys (8.87) and GSH (9.20). Hence it allows Cys to be more reactive than Hcys and GSH since it is more easily deprotonated. Secondly, the reaction between **EuL** and Cys involves intramolecular cyclization in which formation of a seven-membered ring with Cys (**Figure S3**) is generally more kinetically favoured than formation of an eight-membered ring such as with Hcys[16a][16f-h] (**Figure S4**) For GSH, such cyclization reactions are often hindered by the natural bulkiness of the structure itself. (**Scheme 3**) This unique combination in our probe design presents the advantageous discrimination of Cys over Hcys and GSH. The corresponding seven/eight-membered ring was identified by high resolution mass spectra. (**Figure S3** & **S4**)

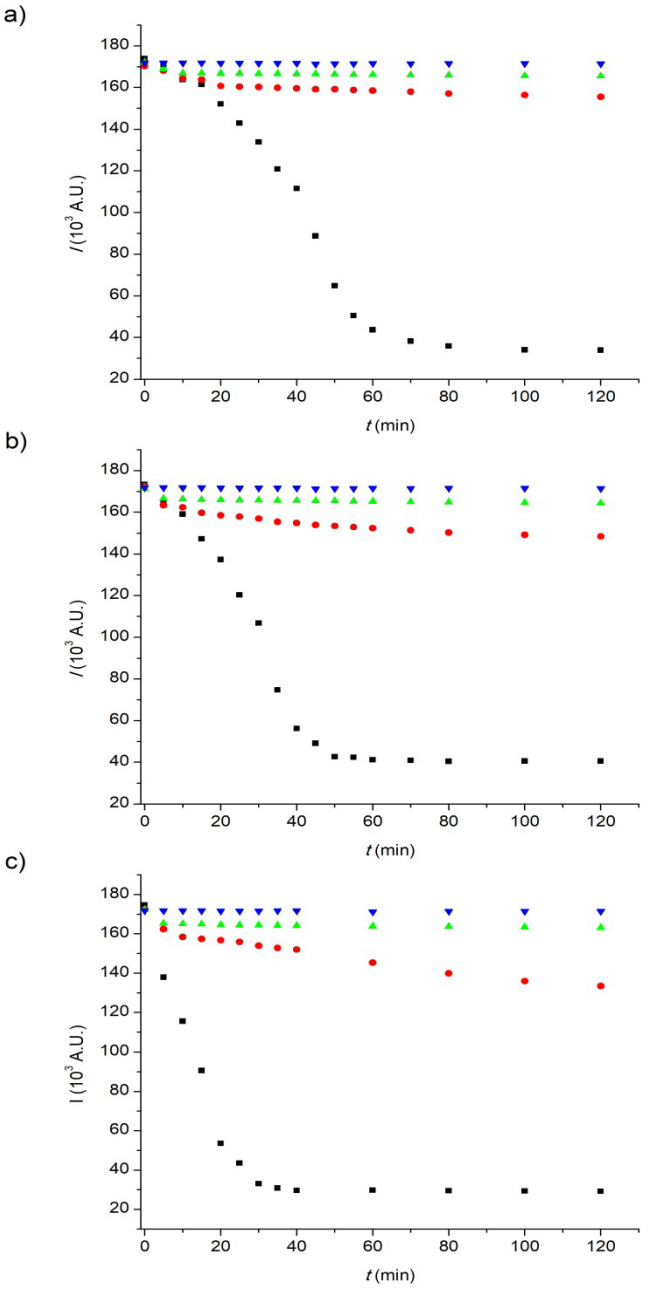
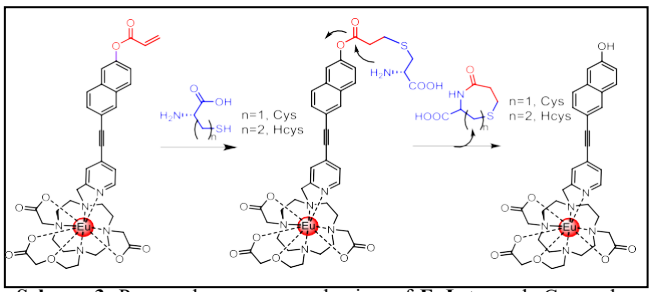


Figure 5. Plots of the luminescent intensity of **EuL** (10 μM) as a function of time in presence of Cys (10 eqv), HCys (10 eqv), GSH (10 eqv) in 0.01 M HEPES (pH 7.4) at **(a)** room temp, **(b)** 37 ^{0}C and **(c)** 45 ^{0}C. Black square: cys, red rhombus: Hcys, green triangle: GSH, blue inverted triangle: Blank.



Scheme 3. Proposed response mechanism of **EuL** towards Cys and Hcys.

Time-dependent luminescent spectra were also analyzed by monitoring the luminescent changes of the reaction mixture in 37 ^{0}C and 45 ^{0}C. (**Figure 5b** and **c**) The luminescent intensity reached a quenching maximum within 55 min (37 ^{0}C) and 40 min (45 ^{0}C).

pH effect towards reaction of EuL with Cys, Hcys and GSH

During the reaction between **EuL** and Cys/Hcys/GSH, the pH value of the reaction mixture was monitored to see if it affect the reaction and hence quenching of the luminescent intensity as ester groups are known to be quite labile and easily attacked by thiol derivative in basic conditions[26]. During these studies, the reaction between **EuL** and Cys, the pH of the reaction medium was tuned to pH 7.4 or 8.0 or 8.4 It is observed that the quenching effect was the highest in these basic regions, whereas when the pH was tuned to the acidic region, pH 5.4, very little quenching in the emission intensity was observed due to less complete reaction in lower pH value. (**Figure 6**) In the case for Hcys and GSH, a similar phenomenon was also detected. (**Figure S13** &**S14**) Based on the result, pH effect is a factor to affect the reaction and hence quenching of the luminescent intensity.

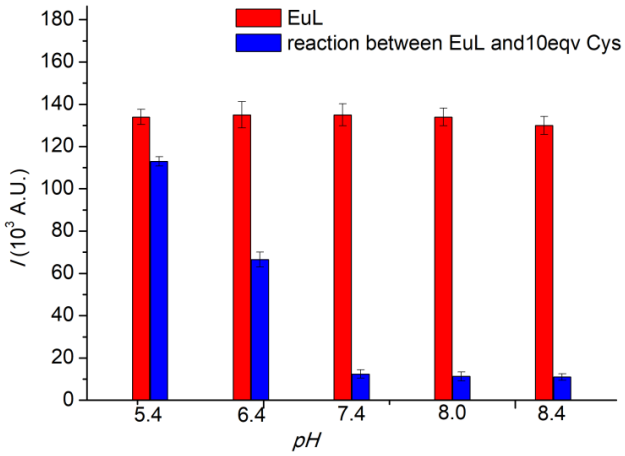


Figure 6. The luminescent intensity of (10μM) **EuL** (red) and reaction of **EuL** with 100 μM of Cys (blue) at different pH values in 80 min.

Solution Stability of EuL: pH dependent study

The luminescent responses of **EuL** and **Eu7** at different pH conditions were investigated to show how the luminescent responses were varied by the pH changes. The measurement was performed after equilibrating for an hour. Luminescent intensity of **EuL** was stable from pH 4 to 8. After pH 8, its emission was quenched. It was proposed that there was basic hydrolysis of the arcrylate group which was found in MS spectrum and q value was zero between pH 8 and 9. For **Eu7,** luminescent responses were stable from pH 4 to 7 and slightly quenched from pH 7 to 9. (**Figure 7**)

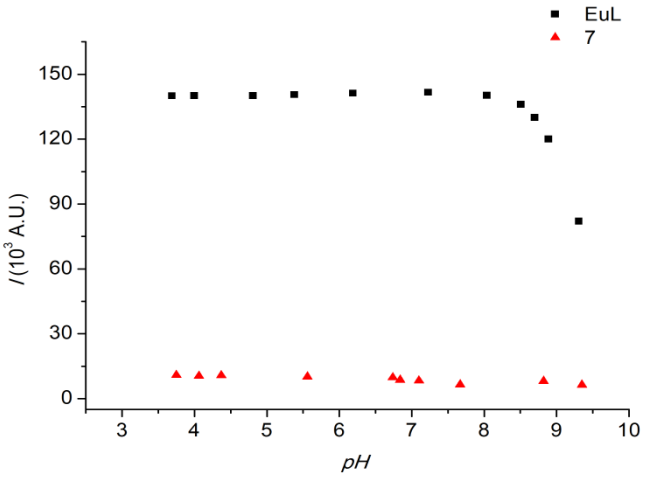


Figure 7. The luminescent intensity of **EuL** (black) and **Eu7** (red) at different pH values.

Solution Stability of EuL: Competition titrations with DTPA

The stability of the **EuL** was tested with diethylenetriaminepentaacetic acid (DTPA) a strong chelator and competitor with pM value 19.04[27]. **EuL** and DTPA were mixed with incremental ratio of DTPA per Eu complex from 1:0 and 1: 900 and then shaken for seven days. The luminescent intensities were then measured. It was observed only around 20 % quenching, indicating **EuL** had high stability and the Eu(III) ion was not easily decomplexed.

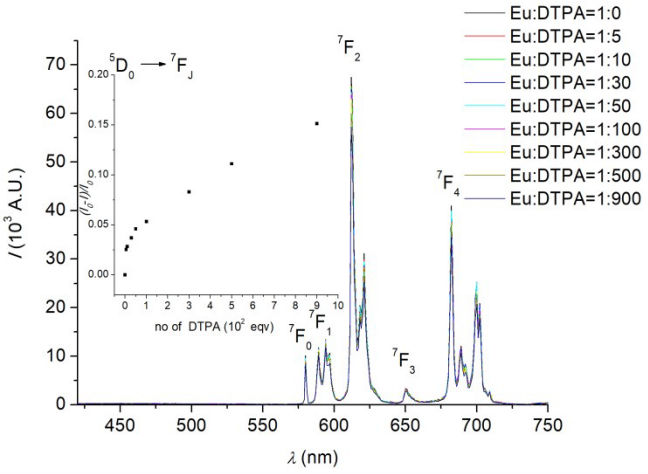


Figure 8. Emission spectra of the **EuL** mixed with different amounts of the DTPA (0.1M HEPES, pH=7.4). Insert: plot of $(I_0\text{-}I)/I_0$ to changing DTPA (λ= 612 nm).

Energy Transfer Process

To interpret the energy transfer processes of **EuL** and **Eu7**, their energy levels of the triplet were calculated by referring to their phosphorescence spectra[28]. The peaks of the phosphorescence of **GdL** and **Gd7** were 505nm ($1.98\times10\hat{}4cm^{-1}$) and 535nm ($1.87\times10\hat{}4cm^{-1}$). （ **Figure S15 & S16** ） According to the experimental results, the schematic energy level diagram depicting the energy transfer process were shown in **Figure S17** & **S18**. The triplet energy levels of **EuL** was higher than the $^5D_0$ level ($1.72\times10\hat{}4cm^{-1}$) of Eu(III) ions with energy gap $2.60\times10\hat{}3cm^{-1}$ while energy gaps for **Eu7** was smaller than $1.50\times10\hat{}3cm^{-1}$. According to Latva's empirical rule, energy gaps $>2.50\times10\hat{}3\ cm^{-1}$ are optimum for the ligand to metal transfer process for Eu(III) ion[29]. Therefore, it demonstrated quenching on titration with Cys.

Conclusions

Water-soluble **EuL** was designed to be highly selective for detection of Cys over other thiol and amino derivatives with a significant quenching of around 90% with a linear relationship correlating the luminescent intensity and the concentration of Cys (5μM to 80μM). According to previous report, the concentration of free Cys in healthy human plasma is approximate 5 μM which is within our desired study range[30]. It was found that the emission of **EuL** was stable from pH 4 to 8. Kinetic studies also indicated that **EuL** had a higher quenching effect by Cys than for both Hcys and GSH. We believe that this work is beneficial to the development of future lanthanide based Cys-targeted probe for selective monitoring of Cys concentrations for potential disease evaluation.

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 (high resolution) and LCQ Deca XP mass spectrometer (low resolution). Elemental analyses were performed on a Elementar Vario EL cube elemental analyzer.

Synthesis of **1**.
(4-bromopyridin-2-yl)methanol (1.0 g, 5.4 mmol) was dissolved in dry triethylamine (10 mL) and dry THF (5 mL) under an atmosphere of nitrogen. Copper(I) iodide (9.8 mg, 0.005 mmol), triphenylphosphine (52 mg, 0.02 mmol) and dichlorobis(tripheny lphosphine)palladium(II) (35 mg, 0.005 mmol) were added to the stirred solution. Ethynyltrimethelsilane (0.79 g, 8.1 mmol)was added in and the mixture was heated to 60 ºC 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 petroleum ether:ethylacetate (PE:EA ） (15:1) to afford **1**. (yield: 46%) ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.74 (s, 9H, CH_3), δ 5.20 (s, 2H, CH_2), 5.91 (s, br, 1H, OH), 7.66 (d, J=4.8, 1H, ArH), 7.92 (s, 1H, ArH), 8.88 (d, J=4.8, 1H, ArH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ0.19, 64.73, 100.54, 102.60, 123.52, 124.79, 132.44, 148.85, 161.07. MS (ESI). Calcd for $C_{11}H_{16}NOSi$ [(M + 1)$^+$] m/z 206.10. Found: m/z 206.12.

Synthesis of **2**.
1 (0.6 g, 2.9 mmol) and Tetra-n-butylammonium fluoride (TBAF) (1.14g, 4.35mmol) were stirred in THF (8 mL) under an atmosphere of nitrogen for 5 h. The combined filtrates were evaporated under reduced pressure, and the crude product was purified by silica

column chromatography eluting with PE:EA (12:1) to afford **3**. (yield: 82 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.36 (s, 1H, CH), δ 4.74 (s, 2H, CH_2), 7.22 (d, J=5.2, 1H, ArH), 7.45 (s, 1H, ArH), 8.44 (d, J=4.8, 1H, ArH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ64.16, 80.95, 82.21, 123.18, 124.58, 128.61, 148.42, 160.51. MS (ESI). Calcd for C_8H_7NO [(M + H)$^+$] m/z 134.06. Found: m/z 134.20.

Synthesis of **3**.
2 (0.4 g, 0.30 mmol) was dissolved in dry triethylamine (8 mL) and dry THF (5 mL) under an atmosphere of nitrogen. Copper(I) iodide (9.8 mg, 0.005 mmol), triphenylphosphine (50 mg, 0.02 mmol) and dichlorobis (triphenylphosphine)palladium(II) (30 mg, 0.005 mmol) were added to the stirred solution. (4-(2-(6-(tert-butyldimethylsilyloxy)naphthalen-2-yl)ethynyl)pyridin-2-yl) metha nol (117 mg, 0.30 mmol) was added in and the mixture was heated to 60 ºC for 12 h. After cooling, the formed precipitate of triethyl amine 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 (5:1) to afford **1**. (yield: 46 %)^{1}H NMR (400 MHz, $CDCl_3$) δ 0.16 (s, 6H, CH_3), δ 0.92 (s, 9H, CH_3), 4.67 (s, 2H, CH_2), 7.27 (d, J= 7.2Hz, 2H, ArH), 7.07-7.18 (m, 2H, ArH), 7.39-7.40 (m, 1H, ArH), 7.51(m, 2H, ArH), 7.88 (s, 1H, ArH), 8.38 (d, J= 5.2Hz, 1H, ArH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ-3.94, 18.62, 26.05, 64.54, 86.91,95.36, 115.30, 117.36, 122.92, 123.35, 124.50, 127.34, 128.90, 129.89, 131.50, 132.47, 132.88, 135.00, 148.84, 155.15, 160.26. MS (ESI). Calcd for $C_{24}H_{27}NSiO_2$ [(M + 1)$^+$] m/z 390.19. Found: m/z 390.26.

Synthesis of **4**.
3 (0.2 g, 0.05 mmol), methanesulfonyl chloride (0.07 g, 0.68 mmol) and triethylamine (0.14 ml, 1.03 mmol) were stirred in DCM (15 ml) under reflux overnight. The reaction mixture was filtered and purified by silica column chromatography eluting with DCM:MeOH (30:1) to afford **4**. (yield: 48 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.27 (s, 6H, CH_3), 1.03 (s, 9H, CH_3), 3.13 (s, 3H, CH_3), 5.36 (s, 2H, CH_2), 7.13 (d, J= 2.4Hz, 1H, ArH), 7.18-7.19 (m, 1H, ArH), 7.42 (d, J= 4Hz, 1H, ArH), 7.51-7.53 (m, 1H, ArH), 7.61 (s, 1H, ArH), 7.68-7.74 (m, 2H, ArH), 8.03 (s, J= 5.2 Hz, 1H, ArH), 8.60(d, 1H, ArH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ 3.89, 18.68, 26.09, 38.51, 71.22, 86.44, 95.11, 115.36, 117.08, 123.49, 124.65, 125.87, 127.46, 128.88, 129.99, 132.76, 133.92, 135.23, 14.66, 154.01, 155.39. MS (ESI). Calcd for $C_{25}H_{29}NSSiO_4$ [(M + 1) $^+$] m/z 468.17. Found: m/z 468.56.

Synthesis of **6**.
4 (0.12 g, 0.26 mmol), **5** (0.12 g, 0.26 mmol) and K_2CO_3 (35 mg, 0.26 mmol) were stirred in ACN (8 ml) under an atmosphere of nitrogen for 15 h .The reaction mixture was filtered and purified by silica column chromatography eluting with gradient from DCM to DCM:MeOH (5:1) to afford **6**. (yield: 52 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.20 (s, 6H, CH_3), 0.95 (s, 9H, CH_3), 1.18-1.20 (m, 9H, OCH_3), 2.35-4.50 (m, 31H, CH_2), 7.04-7.11 (m, 3H, ArH), 7.27 (s, 1H, ArH), 7.42-7.45 (m, 1H, ArH), 7.60-7.67 (m, 3H, ArH), 7.94 (s, 1H, ArH), 8.25 (d, J=5.2, 1H, ArH) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ-4.36, 14.10, 18.20, 25.62, 39.44, 49.60, 50.29, 52.05, 55.91, 58.91, 60.85, 61.12, 67.64, 68.38, 86.01, 95.65, 114.87, 123.01, 125.48, 127.00, 128.39, 128.54, 129.50, 132.15, 132.70, 134.69, 149.21, 154.88, 158.46, 170.28, 172.94. MS (ESI). Calcd for $C_{46}H_{67}N_5O_8Si$ [(M + 1)$^+$] m/z 846.48. Found: m/z 846.60. Elemental analysis calcd (%) for $C_{46}H_{67}N_5O_8Si \cdot 2H_2O$: C,62.63;H,8.11;N,7.94; found: C,62.74; H,8.21;N,7.98.

General procedure of synthesis of **Eu7** and **Gd7**.

6 (68 mg, 0.08 mmol) and LiOH (0.01 M) were stirred in THF (2 ml) overnight. The reaction mixture was filtered and washed by DCM:diethyl ether (1:1) to afford white solid which was then dissolved in water and tuned to pH7 and which was reflux with 1 eqv. of europium(III)/gadolinium (III) chloride hexahydrate (4 mg, 0.008 mmol) in 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 MeOH: chloroform to yield **Eu7**/**Gd7**. (yield 56% and 40%)

Eu7: Elemental analysis calcd (%) for $C_{34}H_{38}N_5O_8Eu \cdot 4H_2O$:C, 47.01;H,5.34;N,8.06; found:C,47.19;H,5.41;N,8.20. Retention time (HPLC): = 8.12min. HRMS (+):796.2006 (M+H)$^+$ [$C_{34}H_{39}EuN_5O_8$]$^+$ requires 796.1992. The isotopic distribution matches closely with the simulated spectrum.

Gd7: Elemental analysis calcd (%) for $C_{34}H_{38}N_5O_8Gd \cdot 3H_2O$: C,47.71;H,5.18;N,8.18; found:C,47.82;H,5.29;N,8.25. Retention time (HPLC): = 8.16min. HRMS (+):803.2036 (M+H)$^+$ [$C_{34}H_{39}GdN_5O_8$]$^+$ requires 803.2034.The isotopic distribution matches closely with the simulated spectrum.

General procedure of synthesis of **EuL** and **GdL**.

Eu7/**Gd7** (20 mg, 0.025 mmol) and acryloyl chloride (20 mg, 0.025 mmol) were stirred in dry DCM (2 ml) in ice for a half day. The reaction mixture was filtered and combined filtrates were evaporated under reduced pressure. The reaction mixture was then washed by DCM and re-crystallized in mixture of chloroform:MeOH to afford **EuL**(yield:48%)/**GdL**(yield:32%).

EuL: Elemental analysis calcd(%) for $C_{37}H_{41}EuN_5O_9 \cdot 3H_2O$:C,49.12;H,5.12;N,7.74; found:C,49.20;H,5.26;N,7.82. Retention time (HPLC):=8.41min HRMS(+): 850.2097(M+H)$^+$ [$C_{37}H_{41}EuN_5O_9$]$^+$requires 850.2072. The isotopic distribution matches closely with the simulated spectrum.

GdL: Elemental analysis calcd (%) for $C_{37}H_{40}GdN_5O_9 \cdot 3H_2O$: C,48.83;H,5.09;N,7.70; found:C,48.94;H,5.16;N,7.81. Retention time (HPLC):= 8.46 min. HRMS(+): 857.2158(M+H)$^+$ [$C_{37}H_{41}GdN_5O_9$]$^+$ requires 857.2155. 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 **Table1**.

Table1. The solvent gradient program in HPLC

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 after 80min 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

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

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.

Selectivity Measurements.
The luminescence emission from **EuL** (10 μM) was measured in 10 mM HEPES buffer (pH 7.4, λ_{ex}=350 nm), with addition of 10 eqv of thiol derivatives such as Hcys, GSH, N-acetyl Cys, cystine, CH_3SCH_3, $NaHSO_3$, H_2S, Na_2SO_4, $Na_2S_2O_3$ and amino acids such as histidine, valine, isoleucine, alanine, arginine, asparagines, aspartic acid, lysine, methionine, serine after 80min at room temp. Then, identical solutions were prepared with the addition of 10eqv Cys to solution of **EuL** and thiols or amino acids and luminescence emission was measured after 80min.

Kinetic studies. [31]
EuL with Cys, Hcys and GSH in 10 mM HEPES buffer (pH 7.4, λ_{ex}=350 nm) at room temperature, 37 ^{0}C and 45 ^{0}C was monitored by measuring the fluorescence intensity at 612nm.

pH effect towards reaction.
EuL (10 μM) was mixed with Cys/Hcys/GSH in pH 5.4, 6.4, 7.4, 8.0 and 8.4 and their luminescence emissions were measured after 80 min at room temp.

Solution stability with DTPA.
Several batches of **EuL** (10 μM) solutions in HEPES buffer (pH 7.4, λ_{ex}=350 nm) at room temperature were mixed with the ratio of added DTPA per Eu complex from 1:0 and 1:900 and then shaken for seven days. For each solution, the emission spectrum was measured.

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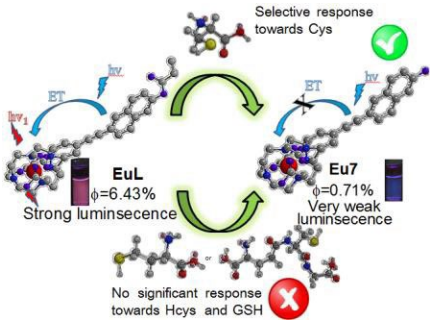
FULL PAPER

Water-soluble **EuL** was highly selective for detection of cysteine (Cys) over other thiol and amino derivatives with a linear relationship correlating the luminescent intensity and the concentration of Cys (5μM to 80μM). **EuL** was stable from pH 4 to 8. Kinetic studies showed that **EuL** had a higher quenching rate with Cys greater than for both Hcys and GSH.



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Reaction Based Europium Complex for Specific Detection of Cysteine over Homocysteine and Glutathione with Variable Temperature Kinetic Studies