

Supplementary Information

A lanthanide based sensor for the time-gated detection of hydrogen sulfide

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General methods

Commercially available solvents and chemicals were used without further purification unless otherwise stated. Routine 300 MHz NMR spectra were recorded on a Varian Mercury 300 spectrometer, ^1H NMR operating at 300 MHz, ^{13}C at 75.5 MHz.

All 500 MHz ^1H Spectra were recorded on a Bruker AVIII 500 spectrometer. All chemical shift (δ) values are given in parts per million.

Low-resolution mass spectra were recorded on a Micromass LCT Premier XE spectrometer. Accurate masses were determined to four decimal places using Bruker μTOF and Micromass GCT spectrometers at the Chemistry Research Laboratory of the University of Oxford. UV-visible spectroscopy was performed using a PG instruments T60 UV/VIS spectrophotometer. Luminescence spectra were measured on a Horiba Jobin Yvon FluoroLog-3 equipped with a Hamamatsu R928 detector and a double-grating emission monochromator. Luminescence lifetimes were obtained tail fit for europium complexes, using a spreadsheet running in Microsoft Excel or using the Fluorescence™ software. Quantum yield were recorded relative to cresyl violet in methanol and Rhodamine 101 in acidified ethanol.

Fluorescence titrations were carried out using a Horiba Fluorolog-3 spectrometer at room temperature. Typically, a 2.0 ml solution of Eu.2 (ca. 1×10^{-5} M) in 10 mM PBS buffer was titrated with a solution of NaHS in the same solvent (ca. 3×10^{-5} M). The added volume was kept below 5% than the initial volume. Emission intensities were corrected to account for dilution.

Synthesis and characterisation

WARNING: organic azides are potentially explosive substances and proper safety precautions should be taken when handling these compounds.

Synthesis of 4-Azidophenacyl bromide (**4**)¹

1-Azidoacetophenone (4.8 g, 29.8 mmol) was dissolved in diethyl ether (15 mL) and dioxane (8 mL) and the mixture stirred in an ice bath while Br₂ was added dropwise (1.5 mL, 29.8 mmol). The reaction was left stirring at RT monitoring its progression by TLC. After typically 3 hours all starting material was consumed. Water was then added to the organic mixture (30 mL) and the aqueous layer was extracted with diethyl ether (3 x 30 mL). The organic solvent was dried with brine, MgSO₄ and evaporated. The crude product was purified by silica gel chromatography (1:1 (v:v) DCM:light petroleum ether) to obtain a pale yellow solid (2.5 g, 35%). ¹H-NMR δ_H(300 MHz; CDCl₃, 298 K) 7.99 (2 H, d, *J*=8.7 Hz), 7.11 (2 H, d, *J*=8.7), 4.40 (2 H, s). ¹³C NMR (76 MHz; CDCl₃): δ 190.4, 146.4, 131.5, 131.0, 119.8, 31.1. IR-ATR(cm⁻¹): 2096 (N₃), 1678 (C=O).

Synthesis of 1,4,7-Tris(*tert*-butoxycarbonylmethyl)-10-(4'-azido-2'-acetophenone)-1,4,7,10-tetraazacyclododecane (**3**)

To a solution of 1,4,7-Tris(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (triester) (0.93 g, 1.56 mmol) in dry acetonitrile (50 ml), potassium carbonate was added (0.43 g, 3.12 mmol) followed by **4** (0.38 mg, 1.56 mmol). This suspension was left stirring at RT under nitrogen for 3 h. The reaction mixture was filtered and the solvent then removed under reduced pressure. The crude product was purified by silica gel column chromatography (DCM/MeOH 90:10) to

obtain an orange solid. (860 mg, 82%). ^1H NMR (300 MHz; CDCl_3): δ 8.09 (d, $J = 8.6$, 2H), 7.21 (d, $J = 8.6$, 2H), 3.91 (s, 2H), 3.20 (m, br, 8H), 3.04 (s, br, 8H), 2.68 (m, br, 4H), 1.45 (s, 27H). ^{13}C NMR (76 MHz; CDCl_3): δ : 206.3, 172.0, 129.86, 129.83, 119.1, 110.8, 81.9, 60.9, 57.1, 56.5, 48.9, 45.4, 27.93, 27.88. HR-ESMS: m/z calc. for $[\text{M}+\text{Na}]^+$ 696.405, found 696.405.

Synthesis of 1,4,7-Tris(carboxymethyl)-10-(4'-azido-2-acetophenone)-1,4,7,10-tetraazacyclododecane (**H_{3.2}**)

TFA (5ml) was added dropwise to a solution of **3** (0.4 g, 0.59 mmol) in DCM (5 ml). The reaction was stirred at RT for 48 h, the solvents were removed under reduced pressure and the product was obtained quantitatively from methanol by trituration with diethyl ether. ^1H -NMR (300 MHz; DMSO-d_6 , 353 K): δ 8.00 (d, $J = 8.8$ Hz, 2H), 7.26 (d, $J = 8.7$ Hz, 2H), 4.63 (s, 2H), 3.68 (m, 6H), 3.20-3.16 (m, 4H), 3.06 (s, br, 12H). ^{13}C NMR (126 MHz; DMSO-d_6): δ 173.0, 158.6, 158.4, 158.1, 157.9, 131.7, 131.5, 130.3, 126.2, 119.53, 119.40, 119.24, 118.6, 116.2, 113.8, 53.60, 53.56, 53.53, 52.07, 52.04, 48.99, 48.81. HR-ESMS: m/z calc. for $[\text{M}+\text{H}]^+$ 506.236, found 506.234.

Synthesis of Eu.2

Europium(III) triflate was added (1.05 eq.) to a solution of the **H_{3.2}** in methanol and the reaction mixture was stirred at 40° C for 30 min. The pH was adjusted to 4 by dropwise addition of an aqueous NaOH solution. The reaction was left stirring at 40° C for 48 h. The methanol was removed under reduced pressure leaving an oil that was dissolved in water. The pH of this solution was adjusted to 10 by addition of aqueous NaOH to remove excess of lanthanide as its insoluble hydroxide. The resulting precipitate was centrifuged and the supernatant filtered. The solvent was removed

under reduced pressure to obtain the product as an orange solid. Yield: 89%. ¹H-NMR (400 MHz; D₂O, pD7): δ 35.6, 28.5, 27.3, 26.3, 8.9, 7.7, -1.7, -3.3, -3.5, -4.3, -5.4, -7.7, -10.0, -10.4, -12.2, -12.9, -13.1, -13.3, -15.6, -16.2, -18.2. Only resolved peaks outside 0 to 5 ppm are reported. HR-ESMS: *m/z* calc. for [M+Na]⁺ 678.116, found 678.117. IR-ATR (cm⁻¹): 2106 (N₃), 1594 (C=O). UV-Vis: λ_{max}: 318 nm (ε = 5700 M⁻¹ cm⁻¹). Luminescence lifetimes: H₂O: 0.60 ms, D₂O: 2.05 ms, q=1.1. Φ = 0.40 % (H₂O).

***In situ* preparation of Eu.1**

Eu.2 (5.6 mg, 8.6 μmol) was dissolved in D₂O (0.7 ml) and NaHS (0.5 mg, 8.6 μmol) was added and the solution was stirred for 30 minutes. This solution was then analysed by HPLC, ES-MS and ¹H-NMR (see below) confirming the formation of Eu.1. ¹H-NMR (400 MHz; D₂O, pD 7): δ 35.6, 30.6, 29.9, 29.0, 6.8, 6.4, 2.2, 0.4, -1.7, -3.0, -3.5, -4.3, -5.4, -7.1, -9.1, -10.4, -13.2, -13.8, -14.2, -15.5, -16.7. Only resolved peaks outside 0 to 5 ppm are reported. HR-ESMS: *m/z* calc. for [M+Na]⁺ 652.125, found 652.125. UV-Vis: λ_{max}: 350 nm (ε = 6300 M⁻¹ cm⁻¹). Luminescence lifetimes: H₂O: 0.44 ms, D₂O: 0.92 ms, q=1.1. Φ = 0.50 % (H₂O).

NMR spectra

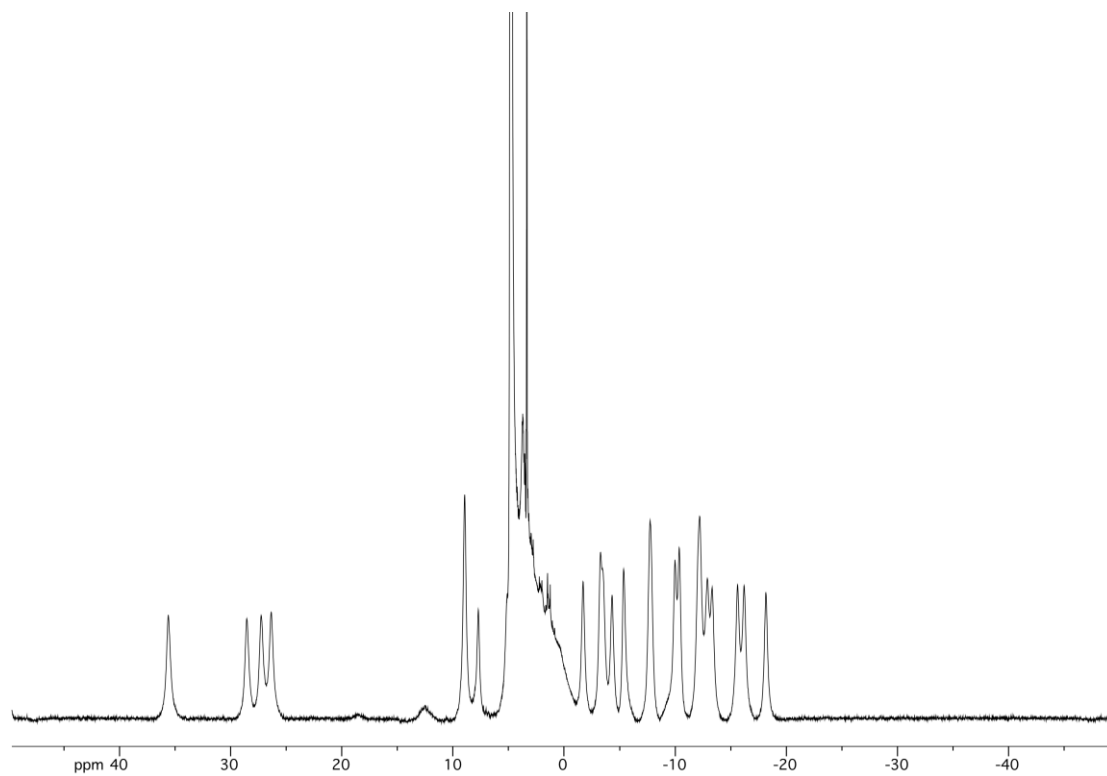


Figure 1. $^1\text{H-NMR}$ of Eu.2 registered in D_2O at pH 7, 298 K.

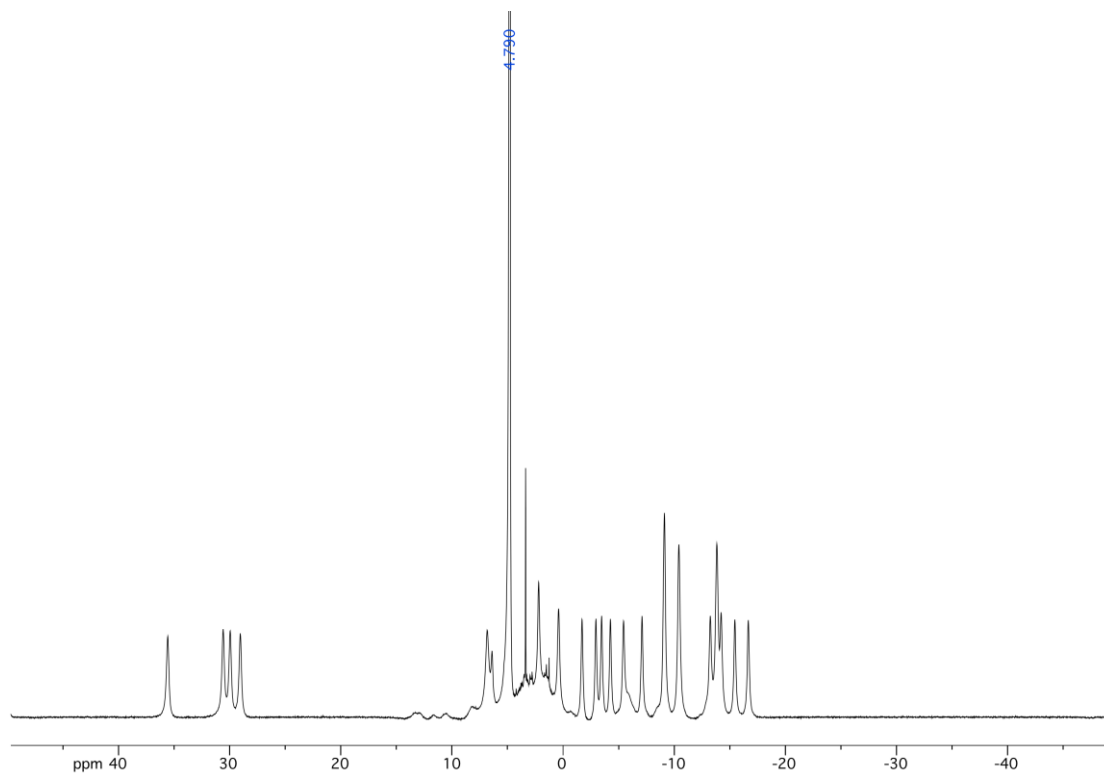


Figure 2. Figure 3. $^1\text{H-NMR}$ of Eu.1 obtained in situ upon reaction of Eu.2 with one equivalent of NaHS in D_2O . Registered in D_2O at pH 7, 298 K.

Solvatochromic studies

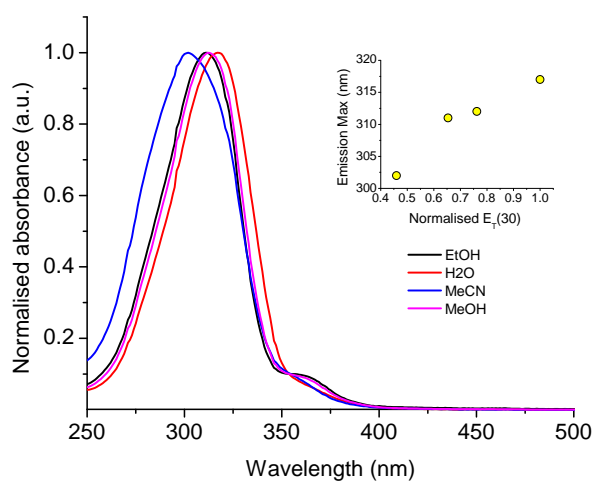


Figure 4. UV-Vis absorption spectra of complex Eu.2 registered in solvent of increasing polarity. Inset: correlation between the maximum of the absorption band the normalized $E_t(30)$ parameter of polarity of the corresponding solvent.

HPLC

HPLC traces were measured using a Gilson analytical HPLC equipped with a Gilson 322 dual pump and a Gilson 152 UV-Vis detector. The stationary phase used was a Discovery Cyano analytical HPLC column and the gradient conditions used are described in the table below.

Time	Acetonitrile (0.1% formic acid)	Water (0.1% formic acid)	Flow rate
0-3	5	95	1
15-25	95	5	1
30-35	5	95	1

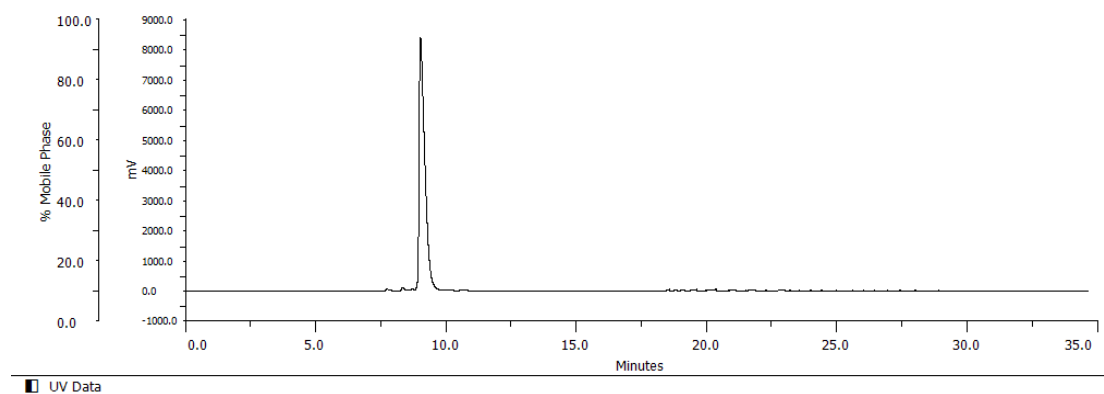


Figure 5. HPLC profile of Eu.2. $\lambda = 320 \text{ nm}$, $t_R = 9.0 \text{ min}$.

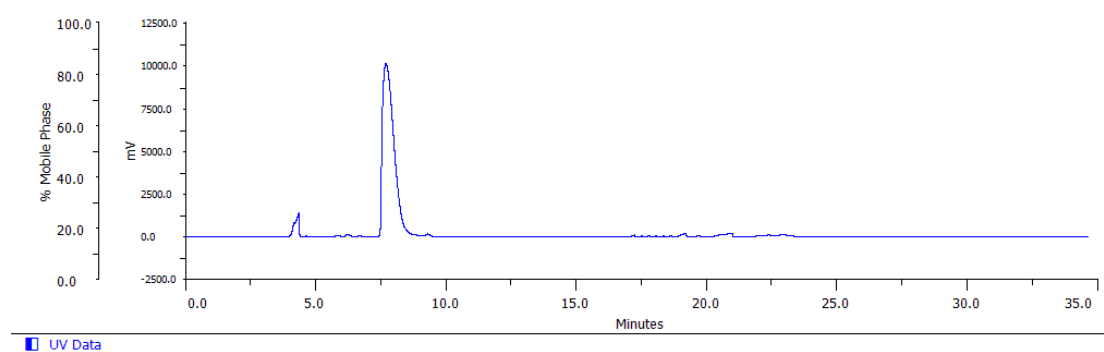


Figure 6. HPLC profile of Eu.2 after reaction with NaHS. $\lambda = 320 \text{ nm}$, $t_R = 7.7 \text{ min}$.

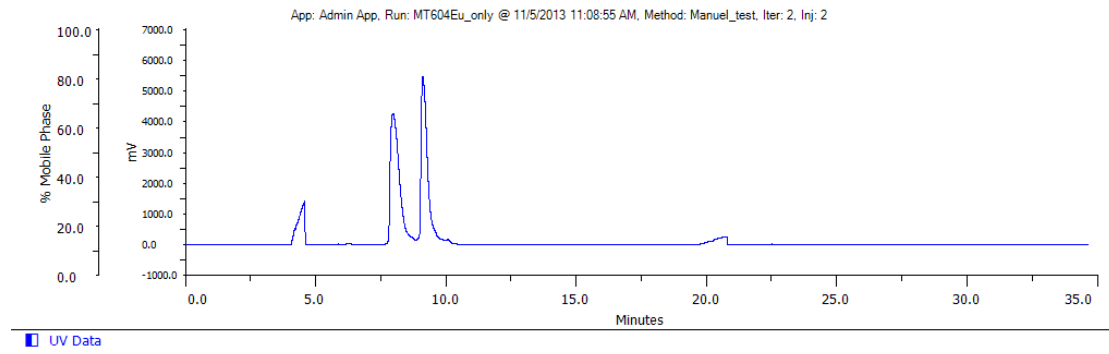


Figure 7. HPLC profile of Eu.2 after reaction with NaHS injected with pure Eu.2. $\lambda = 320$ nm.

Mass spectrometry

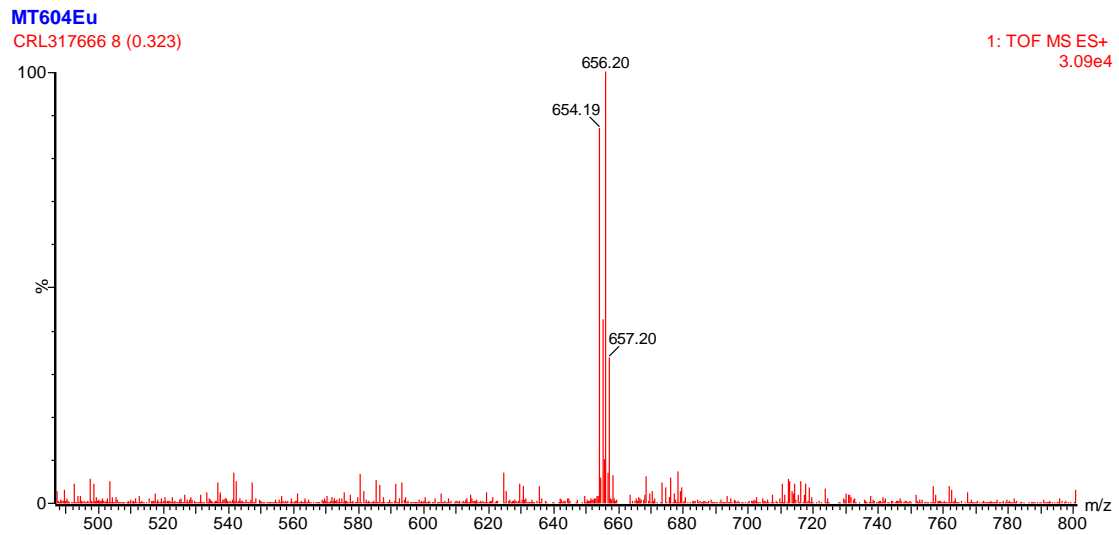


Figure 8. ES-MS of Eu.2

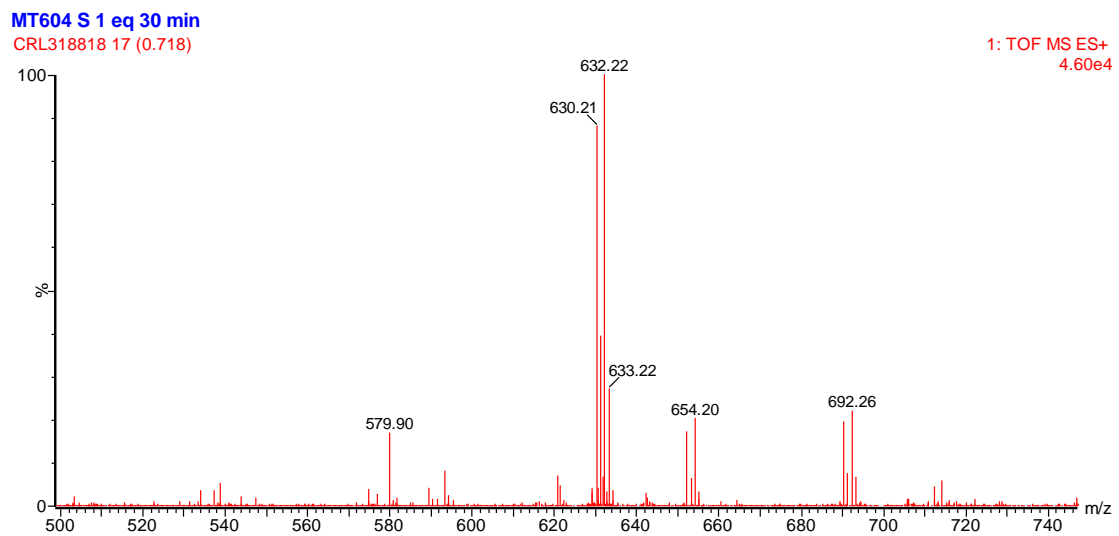


Figure 9. ES-MS of Eu.2 after reaction with 1 equivalent of NaHS in D₂O at RT for 30 minutes.

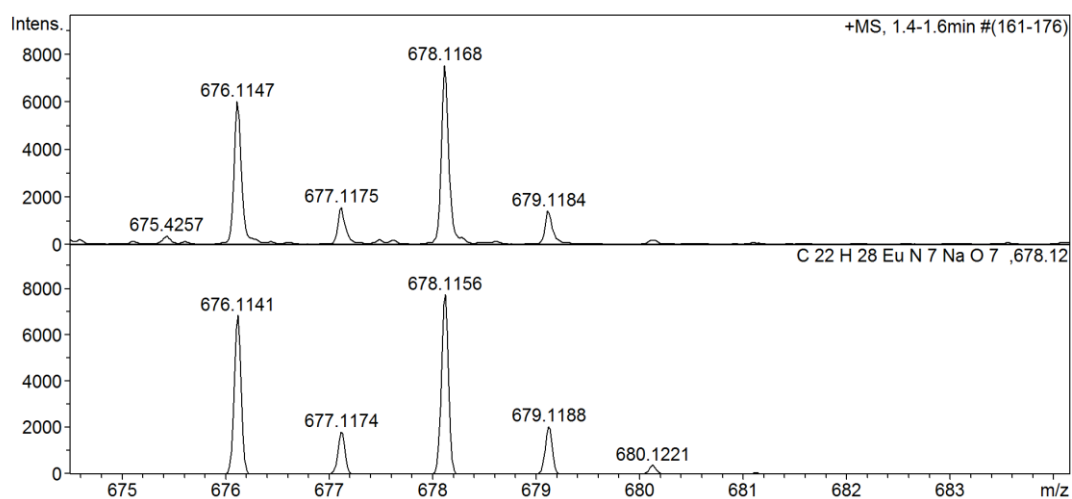


Figure 10. High resolution ES-MS of Eu.2 ($[M+Na]^+$ species).

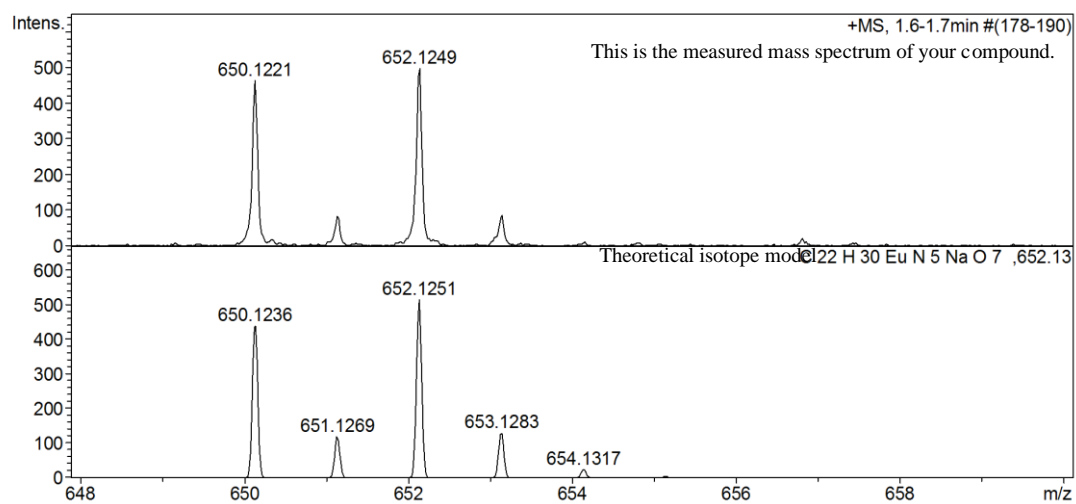


Figure 11. High resolution ES-MS of Eu.1 ($[M+Na]^+$ species).

Fluorescence time response

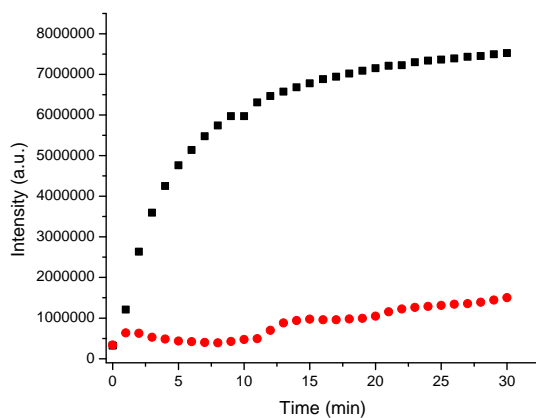


Figure 12. Luminescence time response of Eu.2 (10 μ M) in the presence of 250 μ M NaHS (black squares). Red circles represent the luminescence change of Eu.2 upon irradiation during the same time without addition. Conditions: 10 mM PBS buffer pH 7.4. λ_{ex} = 350 nm.

Detail of fluorescence response of Eu.2 upon addition of competing species

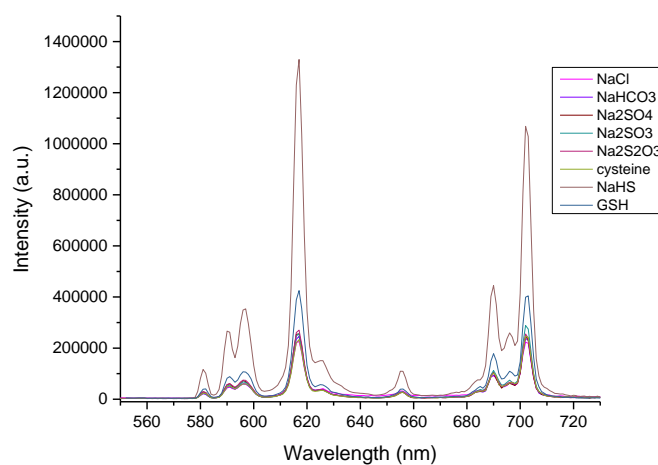


Figure 13. Change in overall luminescence of Eu.2 (10 μ M) upon addition of NaCl (100 mM), NaHCO₃ (25 mM), Na₂SO₄, Na₂SO₃, Na₂S₂O₃, Cysteine, GSH (1 mM) and NaHS (250 μ M) in 10 mM PBS pH 7.4, 295 K.

Main photophysical properties of Eu.1 and Eu.2

	λ_{ex} (nm)	$t_{\text{H}_2\text{O}}$ (ms)	$t_{\text{D}_2\text{O}}$ (ms)	q
Eu.2	320	0.60	2.05	1.1
Eu.1	320	0.44	0.92	1.1

Phosphorescence measurements in serum

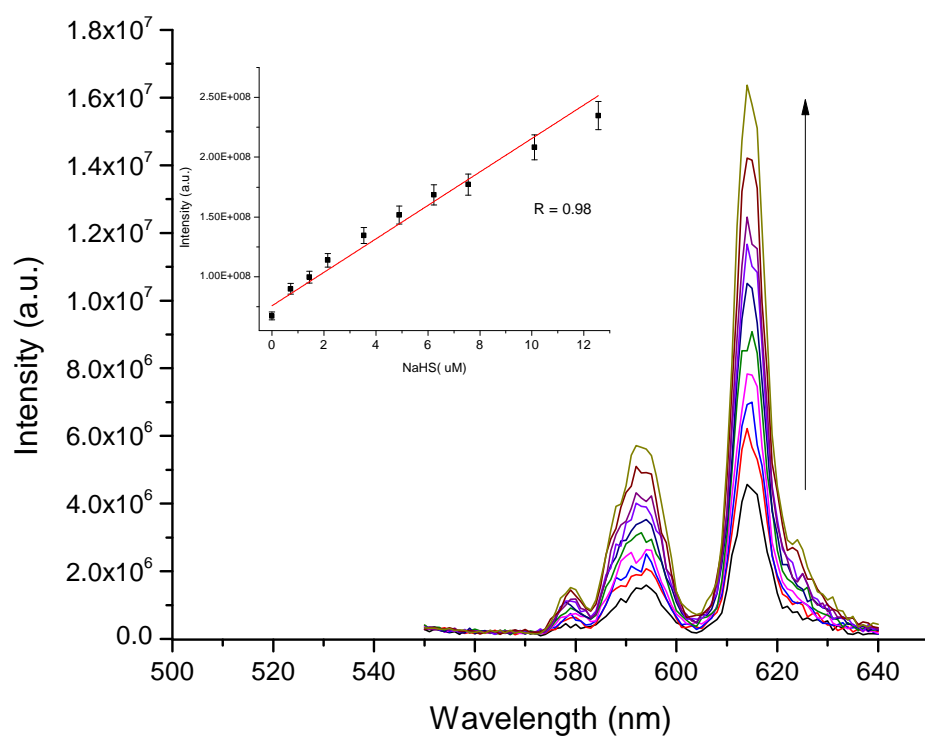


Figure 14. Phosphorescence spectra measured in human serum of Eu.2 (10 uM) upon addition of NaHS. Time delay = 0.05 ms, gate time = 0.2 ms, λ_{ex} = 350 nm.

Fluorescence spectrum of Eu.2 in serum

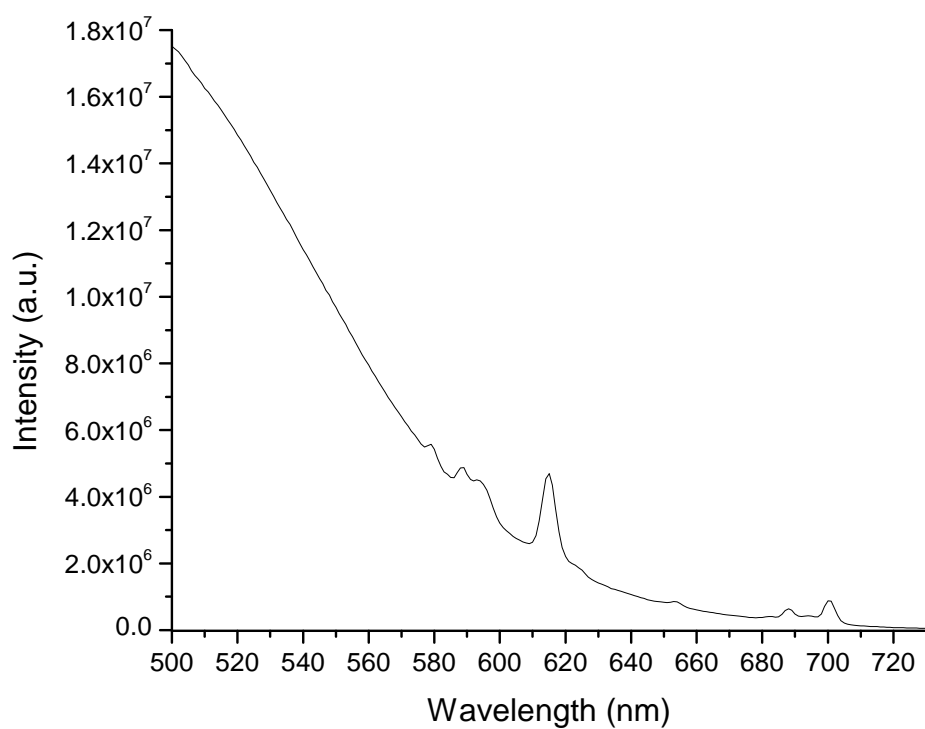


Figure 15 Fluorescence spectrum of Eu.1 (10 μ M) measured in human serum $\lambda_{\text{ex}} = 350$ nm.

References

1. K. Barral, A. D. Moorhouse and J. E. Moses, *Org. Lett.*, 2007, **9**, 1809-1811.