ESI

Chemical Communications 2017

Shuvaev et al

Electronic Supplementary Information

Selectively Switching on Europium Emission

in Drug Site One of Human Serum Albumin

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Materials and Methods

All solvents used were laboratory grade and anhydrous solvents, when required, were freshly distilled over the appropriate drying agent. Water was purified by the 'PuriteSTILLplus' system, with conductivity of $\leq 4 \ \mu S \ cm^{-1}$. All reagents used were purchased from commercial suppliers (Aldrich, Fisher Scientific, Fluorochem) and were used without further purification unless otherwise stated. Reactions requiring anhydrous conditions were carried out using Schlenk-line techniques under an atmosphere of argon.

Optical measurements

Absorption spectroscopy UV/Vis absorption measurements were recorded using a Perkin-Elmer Lambda 900 absorption spectrophotometer, using matched quartz cells.

Luminescence

Emission spectra were measured using a Horiba-Jobin Yvon Fluorolog-3[®] and Horiba-Jobin Yvon Fluoromax-3[®]. The steady-state luminescence was excited by unpolarised light from a 450W xenon CW lamp and detected at an angle of 90° for diluted solution measurements (10 mm quartz cell) by a red-sensitive Hamamatsu R928 photomultiplier tube. Spectra were reference corrected for both the excitation source light intensity variation (lamp and grating) and the emission spectral response (detector and grating). Phosphorescence lifetimes (> 30 µs) were obtained by pulsed excitation using a FL-1040 UP Xenon Lamp. Luminescence decay curves were fitted by least-squares analysis using

Origin[®]. Luminescence quantum yields Q were measured in diluted aqueous solution with an absorbance lower than 0.3, using an integrating sphere. The apparent binding constant of the selected anion was calculated according to equation, using Origin2015[™] software and iterative least squares regression.

$$[X] = \frac{\frac{(F - F_0)}{(F_1 - F_0)} + [Eu]^* (F - F_0)}{K} + [Eu]^* (F - F_0)} (F_1 - F_0)^{-[Eu]^*} (F_0 - F_0)} (F_1 - F_0)^{2}}{1 - (F - F_0)} (F_1 - F_0)}$$

Eu + X \iff EuX $K = \frac{[EuX]}{[X_f][Eu_f]}$

[X]: the total concentration of protein in the solution

[Eu]: the total concentration of the complex

K: the binding constant

F: the ratio of selected peaks

F₀: the ratio at the beginning

F₁: the final ratio

[EuX]: the concentration of the appropriate SA or drug -coordinated complex

 $[X_f]$: the concentration of free SA or drug in the mixture

[Eu_f]: the concentration of the free complex

Circularly Polarised Luminescence

CPL spectra were recorded on a custom built spectrometer consisting of a laser driven light source (Energetiq EQ-99 LDLS, spectral range 170 to 2100 nm) coupled to an Acton SP2150 monochromator (600 g/nm, 300 nm Blaze) allowing excitation wavelengths to be selected with a 6 nm FWHM band-pass. The collection of the emitted light was facilitated (90° angle set up, 1 cm path length quartz cuvette) by a Lock-In Amplifier (Hinds Instruments Signaloc 2100) and Photoelastic Modulator (Hinds Series II/FS2AA). The differentiated light was focused onto an Acton SP2150 monochromator (1200 g/nm, 500 nm Blaze) equipped with a high sensitivity cooled Photo Multiplier Tube (Hamamatsu H10723-20 PhotoSensor red corrected). The detection of the CPL

signal was achieved using the field modulation lock-in technique. The electronic signal from the PMT was fed into the lock-in amplifier (Hinds Instruments Signaloc 2100). The reference signal for the lock-in detection was provided by the PEM control unit. The monochromators, PEM control unit and lock-in amplifier were interfaced with a desktop PC and controlled by Labview2011 code.

Confocal Microscopy

Cell microscopy imaging of the complexes studied in cells was achieved using a custom built epiflorescence microscope (modified Zeiss Axiovert 200M), using a Zeiss APOCHROMAT 63x/1.40 NA objective, combined with a low voltage 365 nm pulsed UV LED focused and collimated excitation source (1.2W). For rapid spectral acquisition, the microscope was equipped at the X1 port with a Peltier cooled 2D-CCD detector (Ocean Optics), used in an inverse 100 Hz time gated sequence. The spectrum was recorded from 400-800 nm with a resolution of 0.24 nm and the final spectrum was acquired using an averaged 10,000 scan duty cycle.

Probe lifetimes were measured on the same microscope platform using a novel cooled PMT detector (Hamamatsu H7155), mounted on the X2 port. Both the control and detection algorithm were written in LabView2014. Time gated images were recorded using a high resolution cooled EO-1312M CCD camera (Thor labs). All duty cycle and gating sequences were established and controlled by in house LabView software⁴.

High resolution Laser Scanning Confocal Microscopy (LSCM) images were recorded on a modified Leica SP5 II microscope, equipped with a new SIM technique called PhMoNa⁴. In order to achieve excitation with maximal probe emission, the microscope was coupled by an optical fibre to a Coherent CW laser (Nd:YAG, 355 nm), operating at 8 mW power. A He/Ne or Ar ion laser was used when commercially available organellespecific stains (e.g. LysoTrackerGreenTM) were used to corroborate cellular compartmentalization.

The microscope was equipped with a triple channel imaging detector, comprising two conventional PMT systems and a HyD hybrid avalanche photodiode detector. The latter part of the detection system, when operated in the BrightRed mode, is capable of improving imaging sensitivity above 550 nm by 25%, reducing signal to noise by a factor

of 5. The pinhole was always determined by the Airy disc size, calculated from the objective in use (HCX PL APO 63x/1.40 NA α Blue), using the lowest excitation wavelength (355 nm). Scanning speed was adjusted to 200 Hz in a unidirectional mode, to ensure both sufficient light exposure and enough time to collect the emitted light from the lanthanide based optical probes (2048 x 2048 frame size, a pixel size of 62 x 62 nm and depth of 298 nm). Spectral imaging on this Leica system is possible with the xy λ -scan function, using the smallest allowed spectral band-pass (5nm) and step-size (3nm) settings. However, much improved spectral imaging in cells was achieved using a custom built and Peltier cooled CCD detector (Ocean Optics, HR2000plus) synchronized to the X1 port.

The europium complexes (10 μ M) were incubated with NIH-3T3 cells for up to 24h to allow complex uptake within the lysosomes; this localisation profile was verified by co-incubation with LysoTrackerGreen.

Live cell cultures studies

A detailed investigation of the cellular behaviour of each complex was conducted using NIH 3T3 mouse skin fibroblast cell using epi-fluorescence and laser scanning confocal microscopy. Initial cell line was sourced from ATCC (NIH 3T3 CRL-1658) and have been established and maintained in a category 2cell culture facility according to established standardized protocol for 12 months; they have been periodically monitored for mycoplasma contamination. Cells were maintained in exponential growth as monolayers in F- 12/DMEM (Dulbecco's Modified Eagle Medium) 1:1 that was supplemented with 10% fetal bovine serum (FBS) or human or goat serum where appropriate. Cells were grown in 75 cm2 plastic culture flasks, with no prior surface treatment. Cultures were incubated at 37 °C, 10% average humidity and 5% (v/v) CO2. Cells were harvested by treatment with 0.25% (v/v) trypsin solution for 5 min at 37 °C. Cell suspensions were pelleted by centrifugation at 1000 rpm for 3 min, and were resuspended in fresh medium by repeated aspiration with a sterile plastic pipette. Microscopy cells were seeded in untreated iBibi 100 µL live cell channels and allowed to grow to 40% to 60% confluence, at 37 °C in 5% CO2. At this stage, the medium was replaced and cells were treated with the studied Eu-complex and co-stains as appropriate,

present in the final imaging medium. For live cell imaging, DMEM/F12 media lacking phenol red was used from this point onwards using a purpose build incubator housing the microscope maintaining 37 °C, 5% CO2 and 10% humidity.

HPLC analysis

HPLC analysis and purification were performed at 295 K using a Shimadzu system (degassing unit DGU-20A5R, Prominence semi-preparative liquid chromatograph LC-20AP, Prominence UV/Vis detector SPD-20A and communications bus module CBM-20A). The solvent system used was ammonium bicarbonate buffer (25 mM, pH = 7) / methanol [isocratic 10 % methanol in buffer (3 min), linear gradient to 100% methanol (10 min), isocratic 100 % methanol (5 min)] or formic acid buffer (0.1%) / Acetonitrile [isocratic 10 % acetonitrile in buffer (3 min), linear gradient to 100% Acetonitrile (10 min), isocratic 100 % acetonitrile (5 min)] flow: 2 ml / min for analytical mode on XBridge C18 column, 4.6 x 100 mm, i.d. 5 μ m.

Electrospray mass spectral analysis and accurate mass determinations

Electrospray mass spectra and accurate masses were recorded on a TQD mass spectrometer or a QTOF Premier mass spectrometer respectively, both equipped with an Acquity UPLC, a lock-mass electrospray ion source and an Acquity photodiode array detector (Waters Ltd, UK), acetonitrile was used as the carrier solvent. Solvent system used for TQD was water (0.1 % formic acid) / Acetonitrile (0.1 % formic acid) [5 % acetonitrile (0.2 min), linear gradient to 95 % Acetonitrile (3.8 min), isocratic 95 % acetonitrile (0.5 min), linear gradient to 5% acetonitrile (0.5 min)], flow: 0.6 ml / min on Acquity UPLC BEH C18 column, 2.1 x 50 mm, i.d. 1.7 μ m. Solvent system used for QTOF was ammonium bicarbonate buffer (25 mM, pH = 7) / Acetonitrile [2 % acetonitrile in buffer (3 min), linear gradient to 40% Acetonitrile (6 min), linear gradient to 100% Acetonitrile (4 min), isocratic 100 % acetonitrile (2 min)] or water (0.1 % formic acid) [linear gradient to 99 % Acetonitrile (5 min), isocratic 99 % acetonitrile (1 min), linear gradient to 100 % water (0.1 min),

isocratic 100 % water (0.9 min)], flow: 0.6 ml / min on an Acquity UPLC BEH C18 column, 2.1 x 50 mm, i.d. 1.7 μ m.

NMR Analysis

¹H and ¹³C NMR spectra were recorded on a Bruker Avance-400 (¹H 400.052 and ¹³C 100.603) spectrometer. Spectra were recorded in commercially available deuteriated solvents. ¹³C and ¹H chemical shift values are quoted in ppm relative to trimethylsilane and all coupling constants are given in Hz. Assignment of the spectra was achieved using COSY, DEPT, HSQC and HMBC experiments. The operating temperature of the spectrometers (usually 295 K) was measured with the aid of an internal calibration solution of ethylene glycol.

Relaxivity measurements were carried out at 310 K, 60 MHz (1.4 T) on a Bruker Minispec mq60 instrument. The mean value of three independent measurements was recorded. The relaxivities of the compounds were calculated as the slope of the function shown in the eqn (1) below,

$$\frac{1}{T_{1,obs}} = \frac{1}{T_{1,d}} + r_1 [GdL^1] \qquad (1)$$

where $T_{1,obs}$ is the measured T_1 , $T_{1,d}$ is the diamagnetic contribution of the solvent (calculated to be 4000 ms) and [GdL¹] is the concentration in mM of the appropriate Gd³⁺ complex (n = 1–6). Errors for all relaxivity values were less than 0.3 mM⁻¹s⁻¹.

The apparent binding constant for the interaction of the Gd^{3+} complexes with Human Serum Albumin (HSA), Bovine Serum Albumin (BSA), iodipamide and ibuprofen was calculated using eq. (2) below:

$$[X] = \frac{\frac{(R-R_0)/(R_1-R_0)}{K} + [GdL^1] \times \frac{R-R_0}{R_1-R_0} - [GdL^1] \times \left(\frac{R-R_0}{R_1-R_0}\right)^2}{1 - \frac{(R-R_0)}{(R_1-R_0)}}$$

$$K = \frac{[Gd \cdot X]}{[X_f][Gd_f]} \tag{2}$$

where [X] is the total concentration of serum albumin in the solution; $[Gd.L^1]$: the total concentration of the complex; *K*: the binding constant; *R*: relaxation rate of a given concentration of *X*; *R*₀: the initial relaxation rate; *R*₁: final relaxation rate; [Gd.X]: the concentration of the serum albumin-coordinated complex; [X_f]: the concentration of free serum albumin in the mixture; [Gd_f]: the concentration of the free complex.

Ligand and Complex Synthesis

The ligand and complex syntheses of $[Ln.L^n]$ (Ln = Eu, Gd; n = 1,2) were carried out as shown in Scheme S1.



Scheme S1

The compounds (4-bromopyridin-2-yl)methanol, trans-1,7,-bis(t-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane (DO2A-^tBu) and 2-(methylsulfonamido)ethyl methanesulfonate were synthesised according to literature procedures described elsewhere. ¹⁻³

Compound 4a



(4-Bromopyridin-2-yl)methanol (0.22 g, 1.18 mmol) and 4-ethynyl-N,N-dimethylaniline (0.20 g, 1.40 mmol) were dissolved in anhydrous THF (10 mL) under argon, followed by addition of pyrrolidine (0.50 mL) and Pd(TPP)₂Cl₂ (0.10 g, 0.14 mmol). The reaction mixture was stirred at 50°C for 24h and the solvent was removed under reduced pressure. The crude material was purified on a silica column (100% DCM to 50%DCM-50%EtOAc), giving a pale brown oil (0.23 g, 77% yield); ¹H NMR (295K, 400 MHz, CDCl₃) $\delta_{\rm H}$ 8.49 (1H, d, ³J_{H-H} = 6.0 Hz, H⁶), 7.44 (2H, d, ³J_{H-H} = 9.0 Hz, H¹⁰), 7.34 (1H, br s, H³), 7.26 (1H, d, ³J_{H-H} = 6.0 Hz, H⁵), 6.67 (2H, d, ³J_{H-H} = 9.0 Hz, H¹¹), 4.77 (2H, s, H¹), 3.03 (6H, s, H¹³); ¹³C NMR (295K, 100 MHz, CDCl₃) 159.1 (C²), 151.0 (C¹²), 148.3 (C⁶), 133.2 (C¹⁰, C⁴), 123.9 (C³), 122.0 (C⁵), 111.7 (C⁹), 108.4 (C¹¹), 96.3 (C⁸), 85.3 (C⁷), 64.1 (C¹), 40.1 (C¹³); *m/z* (HRMS⁺) 253.1344 [M+H⁺]⁺ (C₁₆H₁₇N₂O requires 253.1341).

Compound 7a



The alcohol **4a** (64 mg, 0.25 mmol) was dissolved in anhydrous THF (5 mL) under argon, followed by addition of anhydrous triethylamine (30 μ L, 0.21 mmol) and methanesulfonyl chloride (15 μ L, 0.19 mmol). The reaction mixture was stirred for 2h at rt and the solvent was removed under reduced pressure. The crude material was dissolved in dichloromethane (20 mL) and washed with water (2x10 mL). The organic phase was dried over MgSO₄ and the solvent was removed under reduced pressure. The crude yellow oil was dissolved in anhydrous acetonitrile (5 mL) and was slowly added upon cooling (ice/acetone bath) and vigorous stirring to the solution of DO2A-^tBu (102 mg, 0.26 mmol) and K₂CO₃ (50 mg, 0.36 mmol) in dry acetonitrile (10 mL). The reaction mixture was stirred for 18h allowed to reach rt and was purified using reverse phase HPLC (10% water to 100% water in MeOH over 9 min, 0.1% formic acid). Collected fractions were neutralised using ammonia solution, the solvent was removed under reduced pressure and acetonitrile (20 mL) was added. The insoluble ammonium formate was filtered off and the filtrate was collected. The solvent was removed under reduced pressure to afford a clear oil (60 mg, 37% yield); ¹H NMR (295K, 400 MHz, CDCl₃) $\delta_{\rm H}$ 8.62 (1H, d, ³J_{H-H} = 5.0 Hz, H⁶), 7.44 (2H, d, ³J_{H-H} = 9.0 Hz, H¹⁰), 7.34 (1H, br s, H³), 7.27 (1H, d, ³J_{H-H} = 5.0 Hz, H⁵), 6.68 (2H, d, ³J_{H-H} = 9.0 Hz, H¹¹), 3.73 (2H, s, H¹), 3.04 (6H, s, H¹³), 3.16-2.62 (20H, m, H¹⁴, cyclen core), 1.45 (18H, s, H¹⁷); ¹³C NMR (295K, 100 MHz, CDCl₃) 170.6 (C¹⁵), 157.6 (C²), 150.7 (C¹²), 149.5 (C⁶), 133.2 (C¹⁰, C⁴), 125.2 (C³), 123.9 (C⁵), 111.7 (C⁹), 108.2 (C¹¹), 96.4 (C⁸), 85.3 (C⁷), 81.5 (C¹⁶), 56.6 (C¹), 54.6-46.6 (C¹⁴ and cyclen core), 40.1 (C¹³), 28.2 (C¹⁷); *m/z* (HRMS⁺) 635.4282 [M+H⁺]⁺ (C₃₆H₅₅N₆O₄ requires 635.4285).

Synthesis of the complexes LnL^1 (Ln = Eu, Gd)



The macrocyclic amine 7a (54 mg, 0.09 mmol) was dissolved in dry acetonitrile (10 mL), followed by addition of K₂CO₃ (40 mg, 0.29 mmol) and 2-(methylsulfonamido)ethyl methanesulfonate (17 mg) was added. The oil bath was heated up to 80 °C and the reaction flask was immersed inside the bath. The reaction mixtures was stirred for 18 h and the formation of 8a was confirmed by LC-MS analysis (m/z (HRMS⁺) 756.4459 $[M+H^+]^+$ (C₃₉H₆₂N₇O₆S requires 756.4482)). The solvent was removed under reduced pressure and dichloromethane (15 mL) was added, filtered and the solvent was removed under reduced pressure. The crude was dissolved in methanol (4 mL) and 2.5M NaOH solution (2 mL) was added. The mixture was stirred at 60 °C for 3h. The reaction mixture was neutralised by careful addition of concentrated HCl and LnCl₃•6H₂O (0.2 mmol) was added. The reaction mixture was stirred at 60 °C for 3h and the product was purified using reverse phase HPLC (10% water to 100% water in MeOH over 9 min, (NH₄)₂CO3 buffer (2 g/L), $t_r = 7.6$ min), yielding the desired complex as yellow powder (16 mg for EuL¹ and 18 mg for GdL¹, 24% and 27% yield over 2 steps, respectively); m/z (HRMS⁺) 792.2199 $[M+H^+]^+$ (C₃₁H₄₃N₇O₆S¹⁵¹Eu requires 792.2194), *m/z* (HRMS⁺) 795.2204 $[M+H^+]^+$ (C₃₁H₄₃N₇O₆S¹⁵⁴Gd requires 795.2204); τ_{1r} (GdL¹) = 1.43 mM⁻¹s⁻¹; λ_{abs} (EuL¹) = 365 nm, ϵ (H₂O) = 28000, τ _{H₂O} = 0.30 ms (with HSA).

Compound 4b



(4-Bromopyridin-2-yl)methanol (0.67 g, 3.56 mmol) and 4-ethynylanisole (0.70 mL, 5.40 mmol) were dissolved in anhydrous THF (15 mL) under argon, followed by addition pyrrolidine (0.40 mL) and Pd(TPP)₂Cl₂ (0.25 g, 0.36 mmol). The reaction mixture was stirred at 50°C for 18 h and the solvent was removed under reduced pressure. The crude material was purified on a silica column (100% DCM to 50%DCM-50%EtOAc), giving an orange solid (0.85 g, 100% yield); ¹H NMR (295K, 400 MHz, CDCl₃) $\delta_{\rm H}$ 8.50 (1H, d, ³J_{H-H} = 5.0 Hz, H⁶), 7.48 (2H, d, ³J_{H-H} = 9.0 Hz, H¹⁰), 7.38 (1H, br s, H³), 7.25 (1H, d, ³J_{H-H} = 5.0 Hz, H⁶), 6.90 (2H, d, ³J_{H-H} = 9.0 Hz, H¹¹), 4.76 (2H, s, H¹), 3.83 (3H, s, H¹³); ¹³C NMR (295K, 100 MHz, CDCl₃) 160.4 (C¹²), 159.5 (C²), 148.4 (C⁶), 133.5 (C⁴), 132.6 (C¹⁰), 124.0 (C³), 122.2 (C⁵), 114.2 (C⁹), 114.0 (C¹¹), 94.4 (C⁸), 85.7 (C⁷), 64.2 (C¹), 55.3 (C¹³); *m/z* (HRMS⁺) 240.1027 [M+H⁺]⁺ (C₁₅H₁₄N₂O requires 240.1025).

Compound 5b



The alcohol **4b** (103 mg, 0.43 mmol) was dissolved in anhydrous THF (5 mL) under argon, followed by addition of anhydrous thiethylamine (130 μ L, 0.90 mmol) and methenesulfonyl chloride (55 μ L, 0.70 mmol). The reaction mixture was stirred for 2h at rt and the solvent was removed at reduced pressure. The crude material was dissolved in dichloromethane (20 mL) and washed with water (2X10 mL). The organic phase was

dried over MgSO₄ and the solvent was removed under reduced pressure. The crude yellow oil was dissolved in anhydrous acetonitrile (5 mL) and was slowly added upon cooling (ice/acetone bath) and vigorous stirring to the solution of DO2A-¹Bu (172 mg, 0.43 mmol) and K₂CO₃ (83 mg, 0.60 mmol) in dry acetonitrile (10 mL). The reaction mixture was stirred for 18h allowing to reach rt and was purified using reverse phase HPLC (10% water to 100% water in MeOH over 9 min, 0.1% formic acid). Collected fractions were neutralised using ammonia solution, the solvent was removed under reduced pressure and acetonitrile (20 mL) was added. The insoluble ammonium formate was filtered off and the filtrate was collected. The solvent was removed under reduced pressure giving clear oil (60 mg, 37% yield); ¹H NMR (295K, 400 MHz, CDCl₃) $\delta_{\rm H}$ 8.41 (1H, d, ³J_{H-H} = 5.0 Hz, H⁶), 7.51 (2H, d, ³J_{H-H} = 9.0 Hz, H¹⁰), 7.47 (1H, br s, H³), 7.37 (1H, d, ³J_{H-H} = 5.0 Hz, H⁵), 6.92 (2H, d, ³J_{H-H} = 9.0 Hz, H¹¹), 3.97-2.52 (22H, m br, H¹, H¹⁴, cyclen core), 3.85 (3H, s, H¹³), 3.16-2.62 (20H, m, H¹⁴, cyclen core), 1.48 (18H, s, H¹⁷); ¹³C NMR (295K, 100 MHz, CDCl₃) 160.9 (C¹²), 157.5 (C²), 147.9 (C⁶), 135.2 (C¹⁰), 133.8 (C⁴), 125.4 (C³), 124.9 (C⁵), 114.4 (C⁹), 113.2 (C¹¹), 97.8 (C⁸), 86.6 (C¹⁶), 84.8 (C⁷), 58.7 (C¹, C¹⁴), 55.5 (C¹³), 51.6-43.3 (cyclen core), 28.5 (C¹⁷); *m/z* (HRMS⁺) 622.3965 [M+H⁺]⁺ (C₃₅H₅₂N₅O₅ requires 622.3968).

Complex [EuL²]



The macrocyclic amine **7b** (57 mg, 0.09 mmol) was dissolved in dry acetonitrile (10 mL), followed by addition of K_2CO_3 (25 mg, 0.18 mmol) and 2-(methylsulfonamido)ethyl methanesulfonate (20 mg) was added. The oil bath was heated up to 80 °C and the reaction flask was immersed inside the bath. The reaction mixture was stirred for 18 h and the formation of **8b** was confirmed by LC-MS analysis. The solvent was removed under reduced pressure and dichloromethane (15 mL) was added, filtered and the solvent was removed under reduced pressure. The crude was dissolved in methanol (4 mL) and 2.5M NaOH solution (2 mL) was added. The mixture was stirred at 60 °C for 3h. The reaction mixture was neutralised by careful addition of concentrated HCl and EuCl₃•6H₂O (52 mg, 0.2 mmol) was added. The reaction mixture was stirred at 60 °C for 3h and the product was purified using reverse phase HPLC (0-3 min 10% water, 3-13 min

10% water to 100% water in MeOH, (NH₄)₂CO₃ buffer (2 g/L), $t_r = 10.5$ min), yielding white powder (22 mg, 36% yield over 2 steps); m/z (HRMS⁺) 779.1898 [M+H⁺]⁺ (C₃₀H₄₀N₆O₇S¹⁵¹Eu requires 779.1878); $\lambda_{abs} = 325$ nm; ϵ (H₂O) = 35400; τ (H₂O) = 0.73 (pH = 6), τ (D₂O) = 1.00 (pH = 6); $\phi_{Eu} = 6.6\%$.



Fig. S1 Variation of the relaxivity of $[Gd.L^1]$ as a function of pH, in the presence of 2.2 equivalents of human SA ($pK_a = 6.3$, T = 37°C, 0.1 M NaCl)



Fig. S2 Emission spectra of $[Eu.L^1]$ (10 μ M) in the presence of HSA (0.75 mM) in H₂O (*green*) and D₂O (*blue*), as well as in the presence of bovine SA (0.75 mM) in H₂O (*black*) and D₂O (*red*).



Fig. S3 Emission spectra of $[Eu.L^1]$ (10 μ M) with added human SA (*red*), bovine SA (*orange*) or goat SA (*magenta*) respectively, in the growth medium



Fig. S4 Emission (612 nm) decay curves of the europium ${}^{5}D_{0}$ excited state for [EuL¹] (10 μ M) with either added excess of BSA (*top left*), goat SA (*top right*) or human SA (*bottom*) in the growth medium.



Fig. S5 Variation of the relaxivity of $[Gd.L^1]$ following incremental addition of HSA (log K_b = 3.28). The line shows the fit to the data points using an iterative non-linear least-squares fitting to 1:1 binding model.



Fig. S6 Variation of the relaxivity of $[Gd.L^1]$ following incremental addition of bovine SA (log $K_b = 3.51$). The line shows the fit to the data points using an iterative non-linear least-squares method, fitting to 1:1 binding model



Fig. S7 Variation of the observed emission of $[EuL^1]$ as it is added to HSA (10 μ M, 295K. H₂O). The apparent affinity constant was estimated (log K_b = 5.13), assuming a 1:1 binding model.



Fig. S8 Variation of the emission of $[Eu.L^1]$ (18 µM), following incremental addition of HSA (295K, H₂O). The apparent binding constant of $logK_b = 4.48$ was estimated by assuming a 1:1 binding model.



Fig. S9 Three-dimensional excitation-emission spectra for $[Eu.L^{1}]$ (10 μ M) in the presence of human SA (0.4 mM)



Fig. S10 Circularly polarised emission spectrum of $[Eu.L^2]$ (10 μ M) in the presence of human SA (0.4 mM)



Fig. S11 Emission spectra of $[Eu.L^2]$ (10 μ M) in the presence of human SA (*red*) and bovine SA (*orange*) in the cell growth medium.



Fig. S12 Variation of the lifetime of the excited ${}^{5}D_{0}$ state of [Eu.L²] as a function of pH (p $K_{a} = 4.1$, T = 22°C, 0.1 M NaCl)



Fig. S13 Variation of ratio between ${}^{5}D_{0} \rightarrow {}^{7}F_{1}$ and ${}^{5}D_{0} \rightarrow {}^{7}F_{2}$ transitions of [Eu.L²] as a function of pH (p $K_{a} = 3.8$, T = 22°C, 0.1 M NaCl)



Fig.S14 Variation of the emission intensity and the lifetime of $[EuL^2]$ in cell lysate (p K_a = 7.1, T = 22°C)



Fig.S15 Variation of the observed emission of $[EuL^1]$ (10 μ M) in the presence of HSA (0.4 mM) as a function of added ibuprofen (10 μ M, 295K, PBS buffer)



Fig.S16 Variation of the observed emission of $[EuL^1]$ (10 μ M) in the presence of HSA (0.4 mM) as a function of added iodipamide (10 μ M, 295K, PBS buffer)



Fig.S17 Variation of the observed emission of $[EuL^1]$ (10 μ M) in the presence of HSA (0.4 mM) as a function of added warfarin (10 μ M, 295K, PBS buffer)

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