Electronic Supplementary Information

Enhancing ^{31}P NMR relaxation rates with a kinetically inert gadolinium complex

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Materials and Methods

Commercially available solvents and chemicals were purchased and used without further purification, unless otherwise stated. Anhydrous solvents, where used, were degassed with nitrogen and dried by passing through a MBraun MPSP-800 column. Deionised water was obtained using an Elix®Essential water purification system. Neutral alumina for column chromatography was purchased from Sigma-Aldrich (aluminium oxide activated, neutral Brockmann I, pore size 58 Å, 150 mesh) and soaked in ethyl acetate overnight prior to use. Silica gel for column chromatography was purchased from Merck (Geduran, pore size 60 Å, 230–400 mesh, 40–63 µm). Silica TLC plates were TLC gel 60 purchased from Merck. All NMR spectra were acquired at 298 K. 500 MHz ^{1}H-NMR, 202 MHz ^{31}P-NMR and 126 MHz ^{13}C-NMR spectra were recorded on a Bruker Avance III NMR equipped with an 11.75 T magnet ^{13}C-NMR spectra at 126 MHz were also recorded on a Bruker Avance NMR equipped with a 11.75 T magnet and a ^{13}C detect cryoprobe. The 400 MHz ^{1}H-NMR, 101 MHz ^{13}C-NMR and 162 MHz ^{31}P-NMR spectra were obtained using either a Bruker Avance III HD nanobay NMR equipped with a 9.4 T magnet or a Horizontal 9.4T MR system equipped with a VnmrS Direct Drive2 console (Agilent Technologies, Santa Clara, US) and using a home-built ^{31}P solenoid (inner diameter 5mm, tuned to 161.7MHz). ^{1}H T_1 times were measured using the Horizontal 9.4T MR system with the solenoid coil tuned to proton. All spectra are referenced to the residual solvent peak, unless otherwise stated, and analysed using Bruker TopSpin3.5 NMR software. Mass spectra were acquired using either an Agilent Technology 1260 Infinity or a Waters LCT premier XS. High resolution accurate mass spectra were recorded to 4 decimal places using Bruker µTOF and Micromass GCT systems. Data was processed using Bruker OPUS

software. pH values were measured using a Hanna Instruments pH210 Microprocessor pH Meter with a HI 1131B electrode.

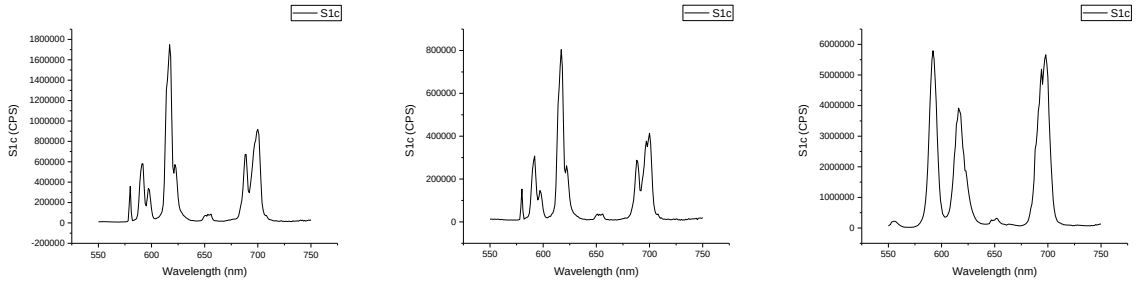
Synthesis

1.Gd and **1.Eu** were synthesised from pDO3A by adaptation of the method reported for other Ln salts.[1] Briefly; pDO3A, (0.2 g, 0.4 mmol, 1 eq.), was dissolved in 3 mL MeOH and the appropriate lanthanide triflate salt added (0.4 mmol, 1 eq.). The reaction was stirred at 60 °C for 30 min and the pH adjusted to pH 5 by addition of 1 M NaOH (aq.) and then stirred for a further 48 h at 60 °C, maintaining the solution at pH 5. The solvent was removed under reduced pressure and the solid dissolved in 2 mL water. Complexes were purified using Amberlite®XAD16N, eluted with 9:1 water/MeCN to remove free lanthanide salts. Purified complexes were tested for the absence of free lanthanide with Xylenol Orange (see below).[2] If free lanthanide remained (*i.e.* the solution was coloured pink), the complex was stirred with Dowex-50 beads and then re-tested.

Characterisation for the novel complex **1.Gd:** HRMS (ES^+) found (*m/z*): 540.10926 (MH^+); $C_{17}H_{26}N_4O_6{}^{158}Gd$ requires 540.10879. (|Δ m/z| = 0.87 ppm). Elemental analysis ($C_{17}H_{25}GdN_4O_6$); calculated. C: 37.91, H: 4.68, N: 10.40; found C: 37.86, H: 4.77, N: 10.35.

Xylenol Orange test

Xylenol Orange was prepared and used in accordance with previous reports.[2] 150 mM acetate buffer at pH 5.8 was prepared by diluting acetic acid (2.87 mL, d = 1.05 $g.mL^{-1}$, 50 mmol) in 800 mL water. The pH was adjusted to 5.8 with 1 M NaOH and the buffer made up to 1 L with water. Xylenol Orange (3 mg, 4.5 µmol) was dissolved in 250 mL acetate buffer to give an 18 µM solution. Aliquots of the solution were frozen immediately for storage and defrosted for use as required. Ln complexes were tested for free Ln^{3+} as follows: 10 µL of 44 mM Ln complex was added to 100 µL Xylenol Orange solution to give a final concentration of 4 mM Ln complex, and the results determined by visual inspection.[i]



Left: emission spectrum of 1.Eu in aqueous solution; Centre: emission spectrum of 1.Eu in aqueous solution in the presence of dissolved $Gd(OTf)_3$ after incubating for three days; Right: emission spectrum of $Eu(OTf)_3$ in water. All spectra recorded following excitation at 397 nm.

Further assessment of the kinetic stability was carried out by using the method of Tóth *et al.*[3] to assess exchange between a solution containing 1.Eu and free gadolinium. As can be seen from the figures above, no change in the form of the emission spectra was observed over three days despite the clear difference between the spectrum of 1.Eu and that of Eu^{3+}(aq).

[i] The method is sensitive enough to determine ≥ 40 µM free Gd^{3+} by eye. Testing solutions of 10–40 mM **1.Gd** therefore allows an estimation of less than 0.1–0.4% free Gd^{3+}.

Sample Preparation for T_1 measurements

Solutions of ATP disodium salt hydrate (Sigma-Aldrich) and PCr disodium salt hydrate (Sigma-Aldrich) in Dulbrecco's Phosphate Buffered Saline (DPBS), Sigma-Aldrich were prepared and adjusted to pH 7.1 – 7.2, with 1 M NaOH as measured by pH meter (HI-2210-02, Hanna Instruments,). Aliquots of the solution were frozen at -80 °C until use. GdDOTA (Dotarem, Guerbet) clinical formulation of 0.5 M was used as received and diluted with distilled water to the specified concentration. The phosphate concentration of all ATP and PCr hydrates were checked by fully-relaxed pulse acquire ^{31}P spectra (TR = 60s, 90° flip angle, 32 averages, 8 kHz bandwidth) (Bruker Avance III HD nanobay NMR spectrometer) and the phosphorus integrals normalised to DPBS (9.57 mM). The contrast agent dilutions were added to the ATP, PCr and DPBS aliquots to give final concentrations of 7.9 mM ATP, 5.5 mM PCr and 8.7 mM DPBS and the appropriate contrast agent concentration (0 – 4 mM).

Relaxation Time Measurements

All measurements were performed at 9.4 T. ^{31}P T_1 times were assessed by inversion recovery (TR > 5* T_1). Samples were measured at room temperature. ^{1}H T_1 was also measured by inversion recovery (TR > 5* T_1). The stability of the phosphate solution was measured overnight by a series of twenty fully relaxed pulse-acquire spectra (TR = 60 s, 90° flip angle, 7.5 µs hard pulse, 60 averages, 512 complex points, 8 kHz bandwidth) (Horizontal 9.4T MR system). Pulse-acquire spectra (TR = 20s, 90° flip angle, 16 averages, 8 kHz bandwidth) of the phosphate solutions with added contrast agent were acquired immediately before the IR experiments. To assess sample stability over the course of the IR measurements, the peak integrals from the pulse-acquire spectra were normalised to that of P_i and compared to the normalised integrals from the final spectrum on the IR curve.

Luminescence methods

Emission and excitation spectra and emission lifetimes were all recorded using a HORIBA Jobin Yvon FluoroLog3 fluorimeter equipped with a double-grating emission monochromator.Lifetime decay curves were fitted using Origin® software. Luminescence lifetimes and q values (obtained using a previously reported method[4]) are given in the table below.

Europium emission lifetimes of Eu.pDO3A in water and PBS, pH 7.4, λ_{ex} = 394 nm.

Complex	τD_2O (ms)	τH_2O (ms)	*q*
Water[a]	1.66	0.39	2.1
PBS[b]	1.73	0.64	0.9

[a][Eu] = 0. 1 mM.

[b][Eu] = 10 µM, [PBS] = 40 mM.

Titration methodology

Concentrated stock solutions of the **1.Eu** complexes in deionised water were made prior to the titration experiments, as well as a separate concentrated stock solution of the phosphate anion (at pH 7.4). For the experiment, the same volume of the stock solution was used for both the initial solution and the titrant solution. Concentrated HEPES buffer was added to both solutions. To the titrant solution an aliquot of the anion solution was added to yield a concentration at least 100 times that of the complex. Both solutions were diluted to the final concentration with deionised water, to give equivalent concentrations of the complex and prevent dilution during the titration. The pH of the final solutions was checked to ensure it was at pH 7.4 (± 0.2).

The initial solution (2 mL) was added to a quartz fluorescence cuvette and small increments of the titrant added with emission spectra recorded at each point.

Determination of binding constants

To determine the binding constants for **1.Eu** with each phosphate species, the data was modelled using a 1:1 binding equation and an association constant K_a determined using equation 1 below:

$$\mathrm{Eu} + \mathrm{X} \leftrightarrow [\mathrm{Eu.X}] \qquad K_a = \frac{[\mathrm{Eu.X}]}{[\mathrm{Eu}][\mathrm{X}]} \tag{1}$$

The integrals of the peak intensity were calculated for changes in quantum number ΔJ = 1 (~560–600 nm), ΔJ = 2 (~600–640 nm) and ΔJ = 4 (~ 670–720 nm), as a function of increasing anion concentration. These values were globally fitted using Dynafit® software[5] and associated binding constants determined.

Example Dynafit® Script:

```
[task]
task = fit
data = equilibria
algorithm = LM
[mechanism]
Eu + PBS <===> complex1 : K1 assoc
[constants]
K1 = 100 ??
[concentrations]
Eu = 1E-5
[responses]
[data]
variable PBS
file ./605-630.txt | response Eu = 4.7E12 , complex1 = 1.4E13 ?
file ./680-710.txt | response Eu = 1.4E12 , complex1 = 4.5E12 ?
file ./580-605.txt | response Eu = 2.6E12 , complex1 = 8.2E12 ?
[output]
directory ./output/
```

Binding isotherms

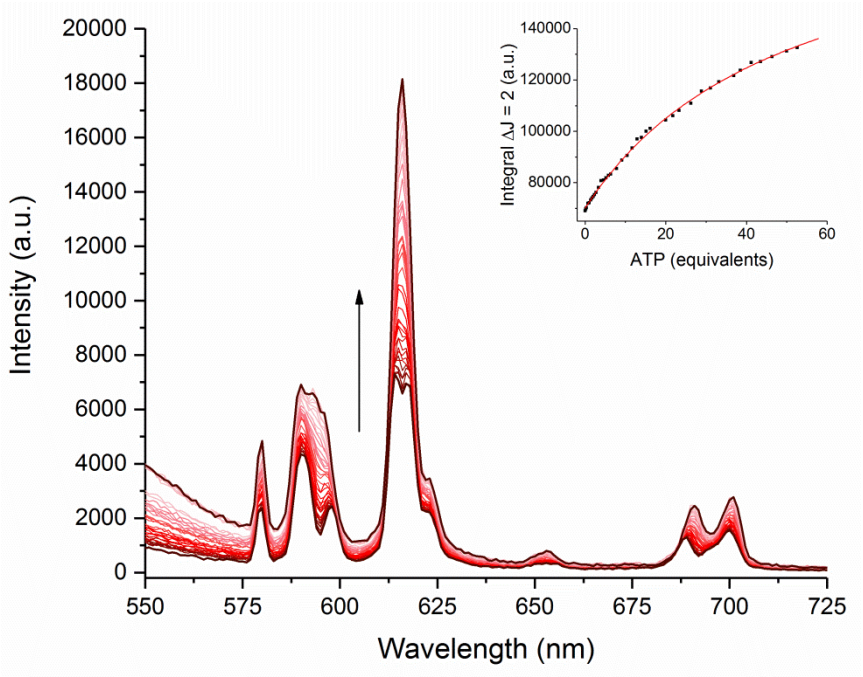


Figure S1: Europium fluorescence of 0.1 mM **1.Eu** in 10 mM HEPES with increasing [ATP]. Insert shows integral of ΔJ = 2 transition with increasing equivalents of ATP, λ_{ex} = 394 nm, pH 7.4.

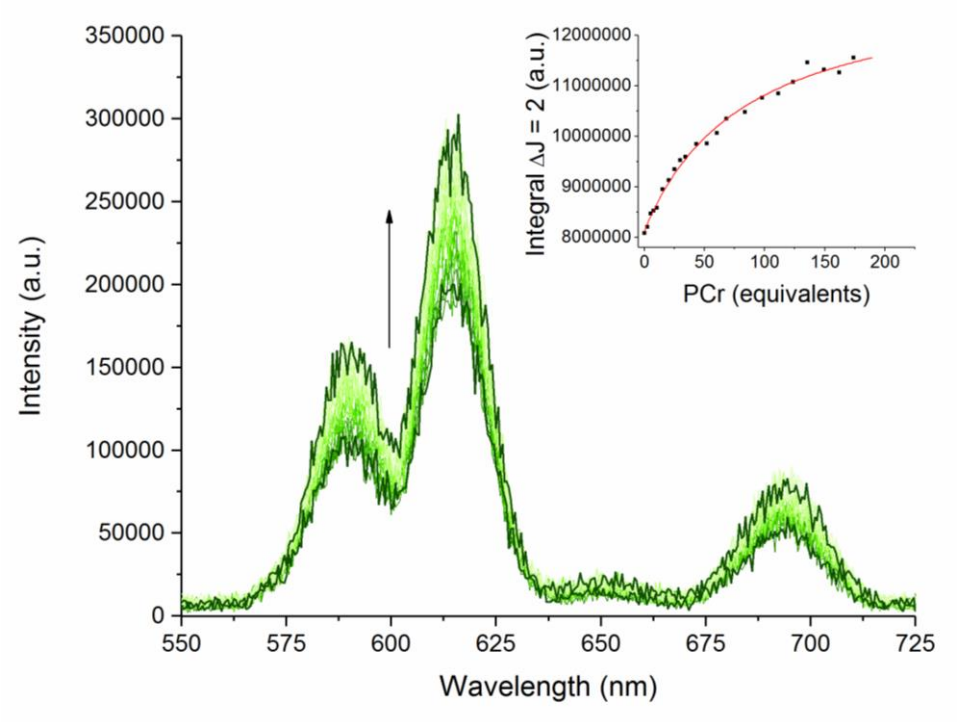


Figure S2: Phosphorescence of 0.1 mM **1.Eu** in 10 mM HEPES with increasing [PCr]. Insert shows integral of ΔJ = 2 transition with increasing equivalents of PCr, λ_{ex} = 394 nm, pH 7.4.

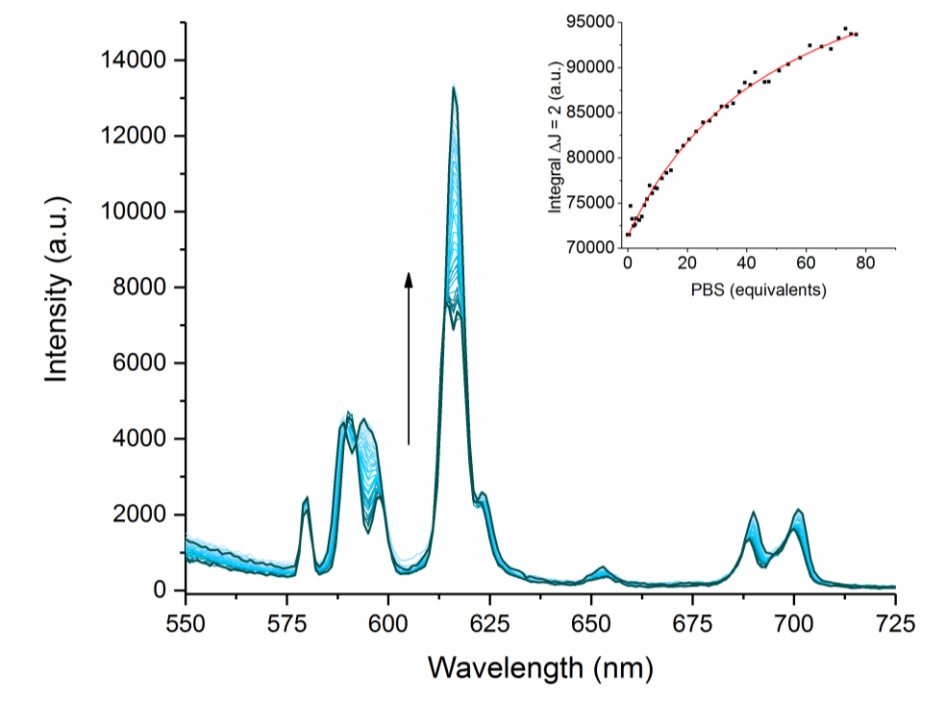


Figure S3: Europium fluorescence of 0.1 mM **1.Eu** in 10 mM HEPES with increasing [PBS]. Insert shows integral of ΔJ = 2 transition with increasing equivalents of PBS, λ_{ex} = 394 nm, pH 7.4.

References

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3 É. Tóth, R. Király, J. Platzek, B. Radüchel and E. Brücher, *Inorg. Chim. Acta*, 1996, **249**, 191.

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