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Accessory Publication

Luminescent terbium contrast agent for microdamage bone detection

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1. Spectrophotometric Titrations

UV-Vis. Spectrophotometer settings

Scan: 200-800 nm

Ex. Slit: 1 nm

Em. Slit: 1 nm

Scan Rate: 600 nm min^{-1}

Luminescence Settings: Fluorescence Emission

Mode: Fluorescence	**Excitation:** 225 nm
Start : 250 nm	**Scan control :** Medium
Stop: 450 nm	**Ex. Slit:** 20 nm
Emission Filter: 250-395 nm	**Em. Slit:** 10 nm
PMT Voltage: 800 V	

Luminescence settings: Lanthanide Luminescence

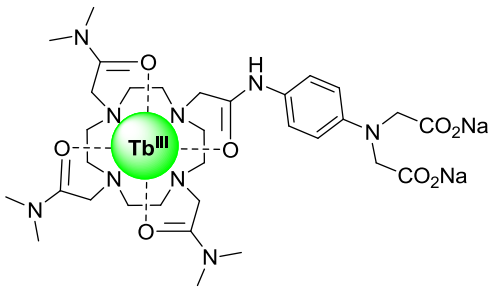
Mode: Phosphorescence	**Excitation:** 285 nm
Total Decay: 0.02 s	**Scan:** 450-650 nm
Flash: 1	**Delay:** 0.1 ms
Gate: 5 ms	**PMT Voltage:** High (900V)
Excitation slit width: 20 nm	**Emission slit width:** 10 nm

Luminescence settings: Lifetime studies

The technique employed to determine the hydration number (*q*) was to measure the excited state lifetimes ($^5D_4$) of the Tb^{III} complex in H_2O (τ_{H2O}) and D_2O (τ_{D2O}), by indirect excitation of the Tb^{III} at 285 nm. The Horrocks modified equation developed by Parker *et al.* was then used to calculate the number of metal bound water molecules. Lifetimes were measured as an average of 5 measurements all agreeing to within 5% of each other. The general settings used are as stated below.

Indirect excitation: 285 nm	**Total Decay:** 30 ms
Emission: Tb^{III} – 545 nm	**Delay** 0.2 ms
No Cycles: 100	**PMT Voltage:** High
Flash: 1	**Emission slit width:** 10 nm
Gate: 0.1 ms	
Excitation slit width: 20 nm	

Complex 1.Tb.Na



The desired product was obtained as a pale yellow solid (0.080 g, 81% yield). M.P. decomposed above 250 °C; HRMS (*m/z*, ES^+): Calculated for $C_{34}H_{53}N_9O_{14}S_2F_6Tb$ *m/z* = 1148.2311 $[M+2(CF_3SO_3)-2Na+2H]^+$. Found *m/z* = 1148.2356; ^1H NMR (400 MHz, D_2O, δ_H): 84.92, 79.51, 69.02, 67.04, 61.52, 58.42, 54.56, 53.79, 52.79, 49.04, 44.07, 44.38, 22.96, 21.53, 20.53, 16.67, 15.77, 14.20, 11.41, 10.37, 8.35, 7.84, 7.19, 6.72, 6.59, 6.43, 6.34, 3.52, 1.30, 1.17, -0.06, -82.05, -85.69, -88.45, -97.73, -101.59, -103.47; IR υ_{max} (cm^{-1}): 2972, 1603, 1438, 1251, 1229, 1168, 1088, 1035, 945, 906, 878, 864, 765, 687, 638.

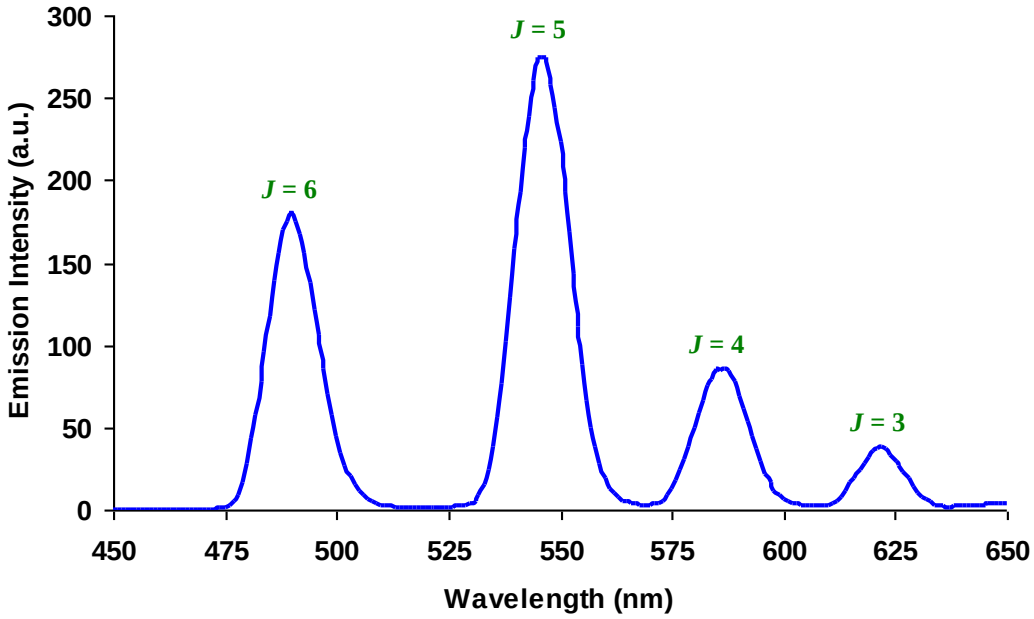


Figure S1: Lanthanide luminescence spectra of **1.Tb.Na** in H_2O (20 mM HEPES, 135 mM KCl, pH 7.4), using an excitation wavelength of 285 nm

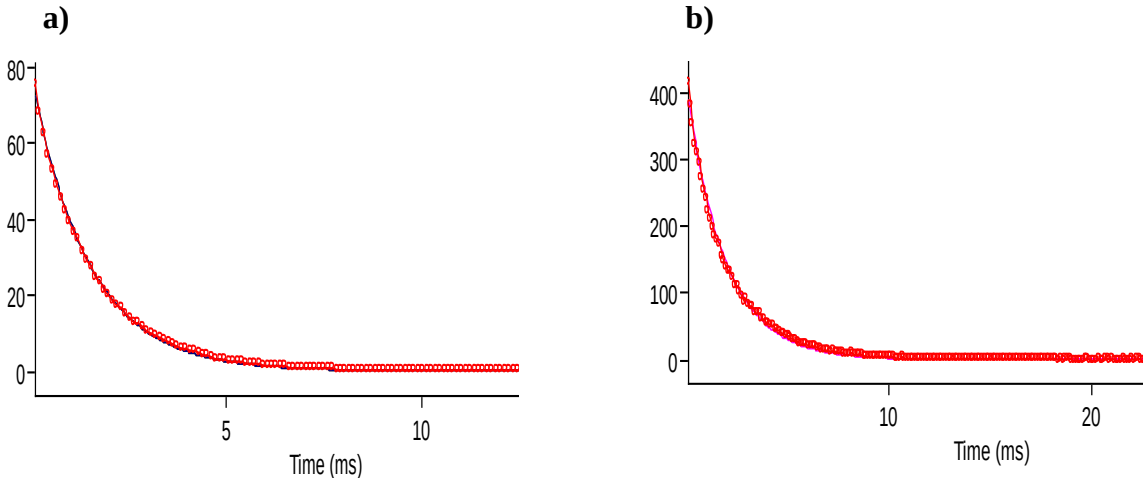


Figure S2. Luminescence decay of **1.Tb.Na** (fit to a mono exponential function) in a) H_2O and b) D_2O.

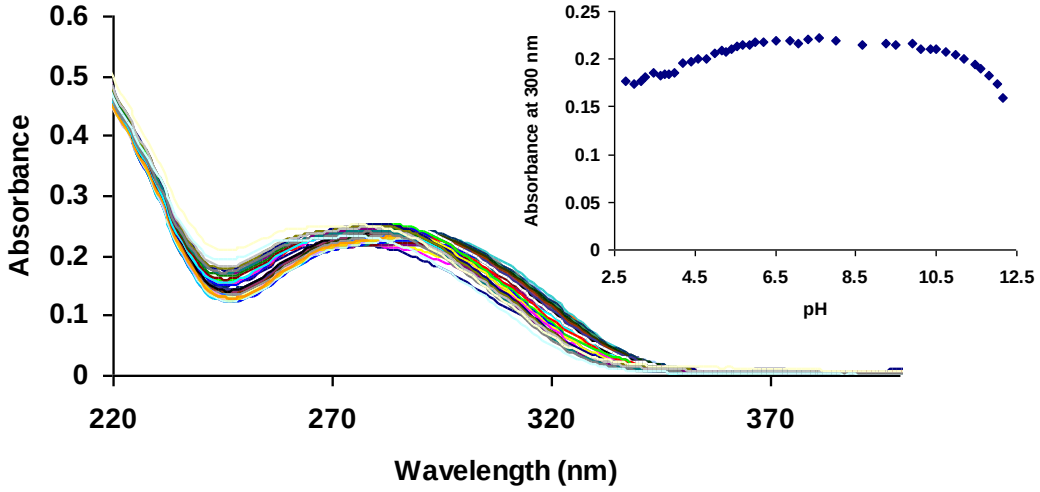


Figure S3a. Absorption spectrum of **1.Tb.Na** as a function of pH [I = 0.1 M NEt_4HClO_4 (TEAP)]. Inset: Absorbance at 300 nm as a function of pH.

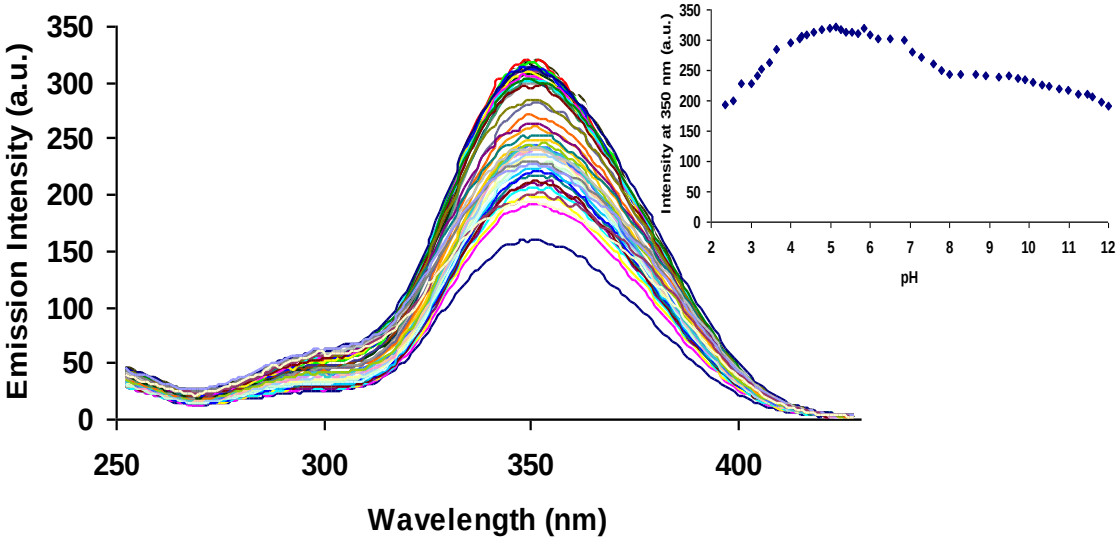


Figure S3b: Fluorescence spectrum of **1.Tb.Na** as a function of pH [I = 0.1 M NEt_4HClO_4 (TEAP)]. Inset: Emission intensity at 350 nm as a function of pH.

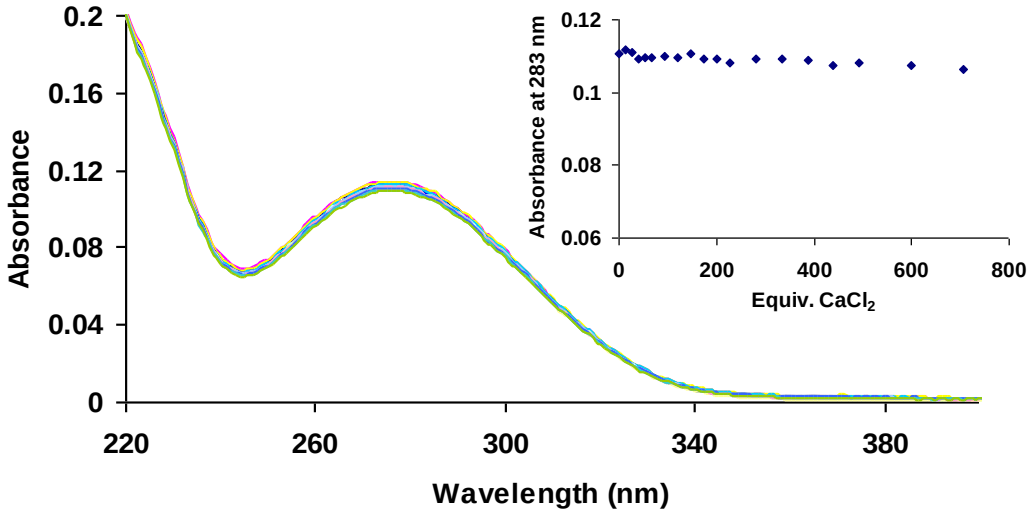


Figure S4a. Changes in the absorption spectrum of **1.Tb.Na** (10 μM) in the presence of $CaCl_2$ (0-7.5 mM) in H_2O. Inset: Plot of absorbance at 285 nm with equivalents of $CaCl_2$ added.

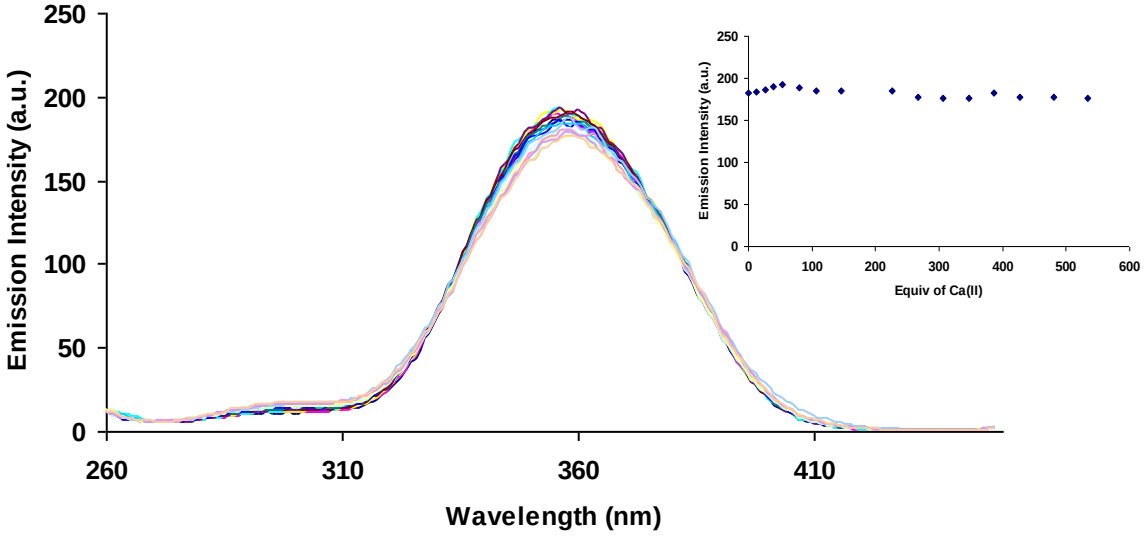


Figure S4b. Changes in the fluorescence of **1.Tb.Na** (10 μM) in the presence of $CaCl_2$ (0-5.5 mM) in H_2O. Inset: Emission intensity at 360 nm with equivalents of $CaCl_2$ added.

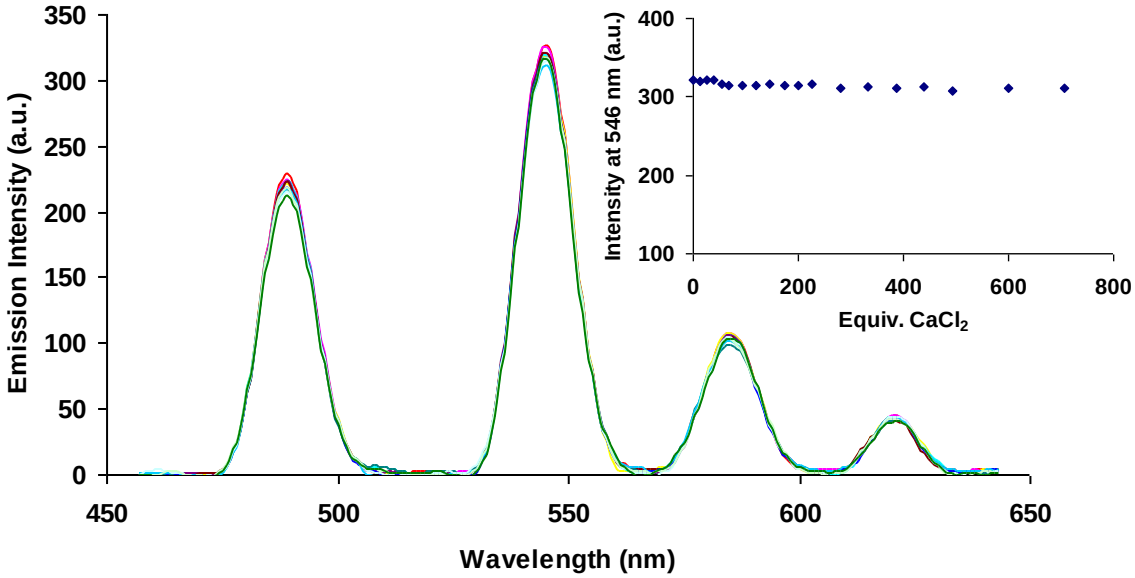


Figure S4c. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of $CaCl_2$ (0-7.5 mM) in H_2O. Inset: Plot of intensity at 546 nm with equivalents of $CaCl_2$ added.

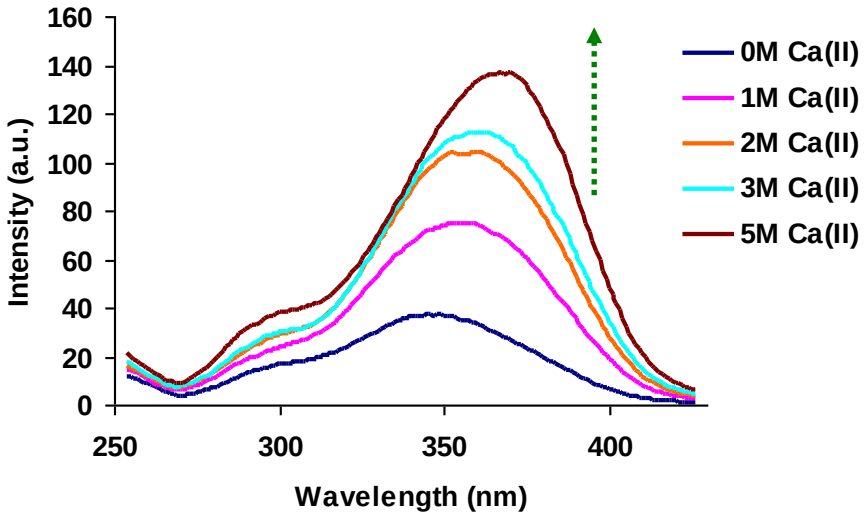


Figure S5a. Changes in the fluorescence of **1.Tb.Na** in the presence of high concentrations of $CaCl_2$ (0-5 M) at pH = 7.4 (0.1 M HEPES, 135 mM KCl).

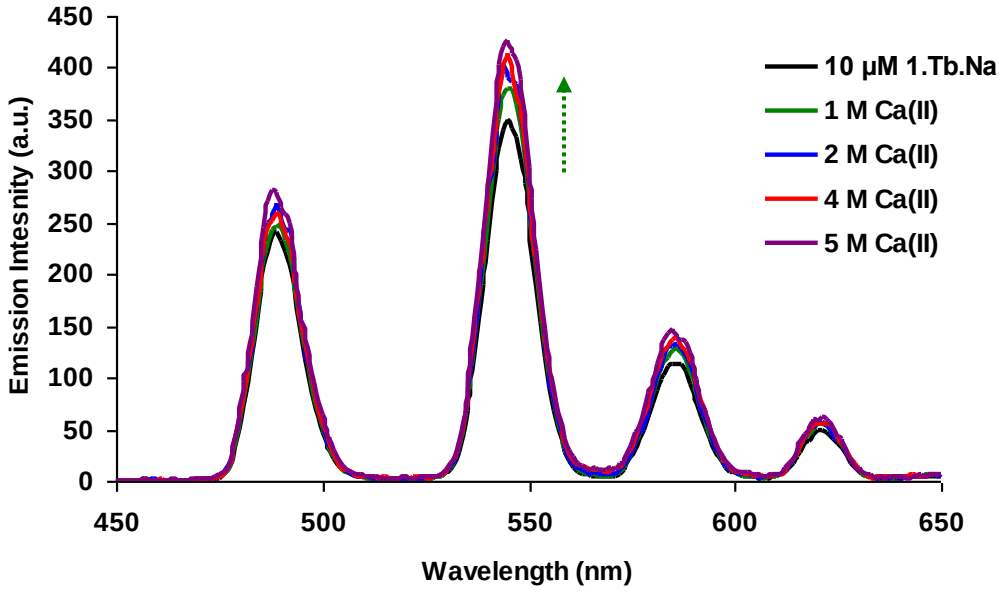


Figure S5b. Changes in the Tb^{III} emission of **1.Tb.Na** in the presence of high concentrations of $CaCl_2$ (0-5 M) at pH = 7.4 (0.1 M HEPES, 135 mM KCl).

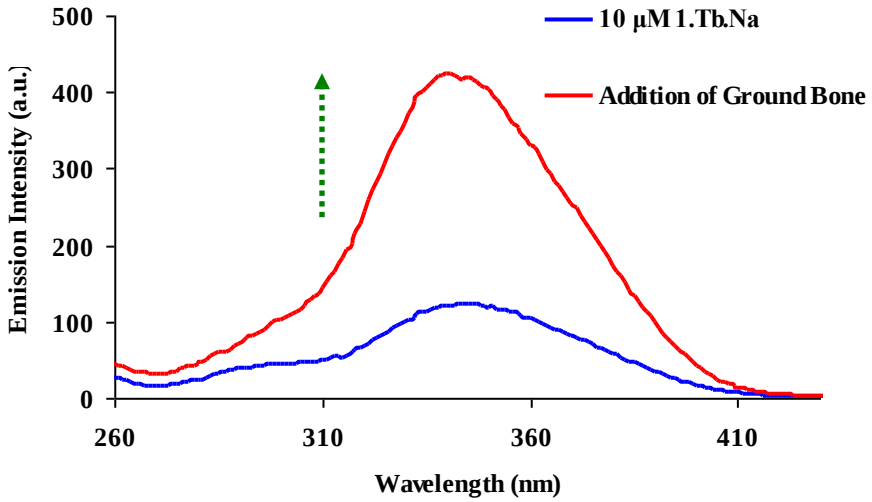


Figure S6a. Changes in the fluorescence of **1.Tb.Na** (10 µM) in the presence of ground bone at pH = 7.4 (0.1 M HEPES, 135 mM KCl).

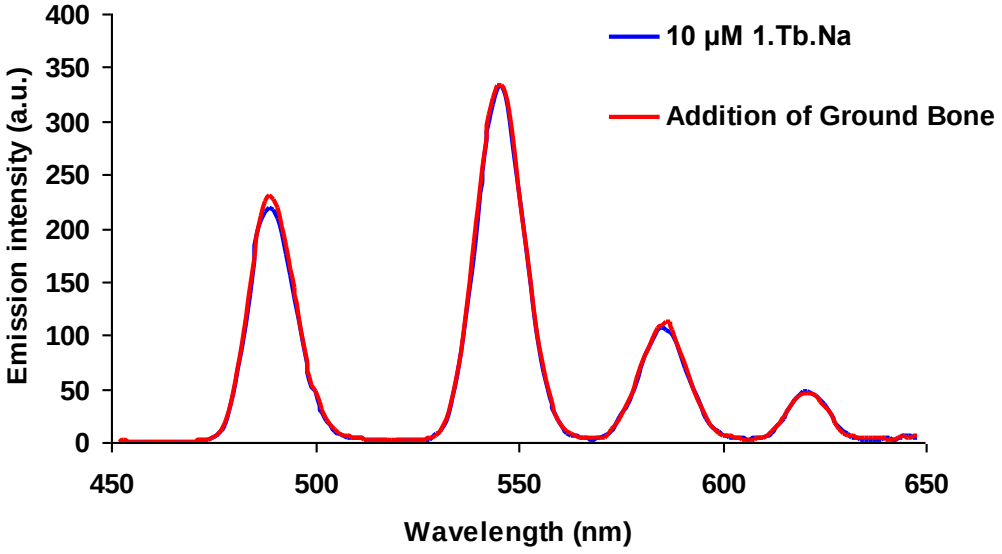


Figure S6b. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of ground bone at pH = 7.4 (0.1 M HEPES, 135 mM KCl).

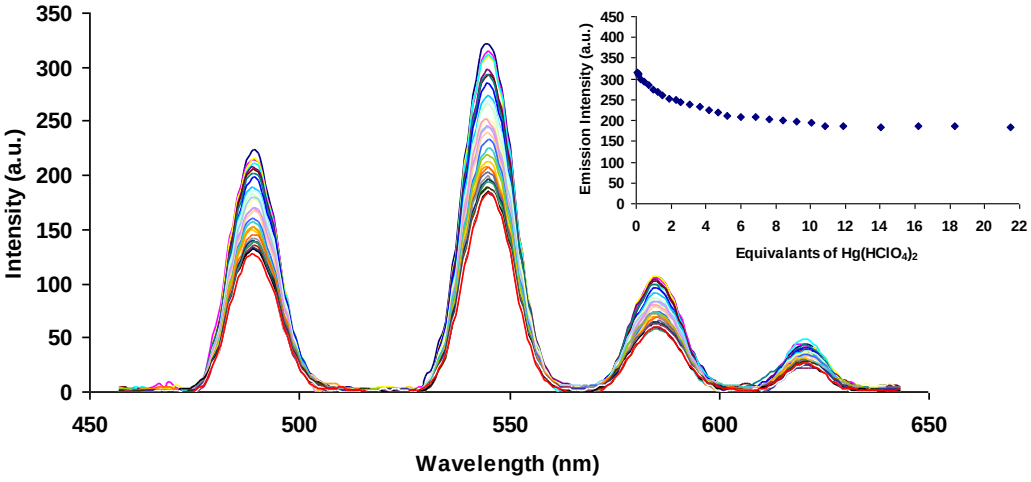


Figure S7a. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of $Hg(HClO_4)_2$ in H_2O. Inset: Plot of intensity at 545 nm with equivalents of $Hg(HClO_4)_2$ added.

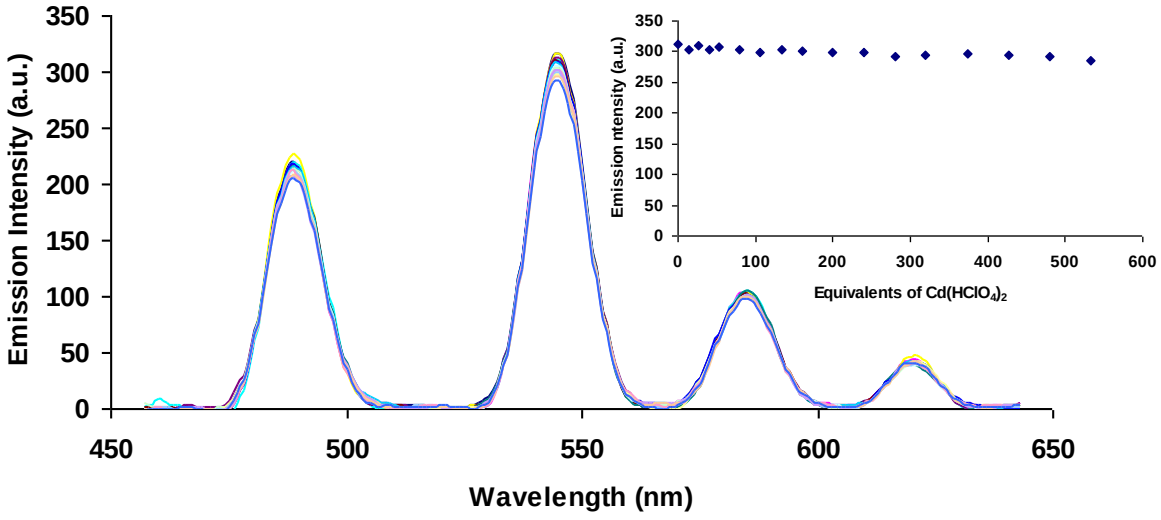


Figure S7b. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of $Cd(HClO_4)_2$ in H_2O. Inset: Plot of intensity at 545 nm with equivalents of $Cd(HClO_4)_2$ added.

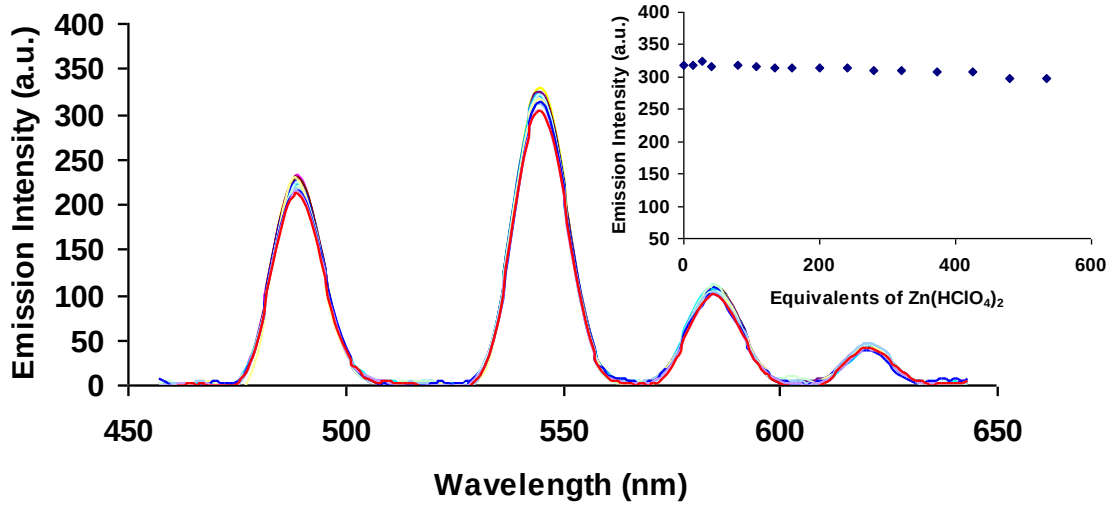


Figure S7c. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of $Zn(HClO_4)_2$ in H_2O. Inset: Plot of intensity at 545 nm with equivalents of $Zn(HClO_4)_2$ added.

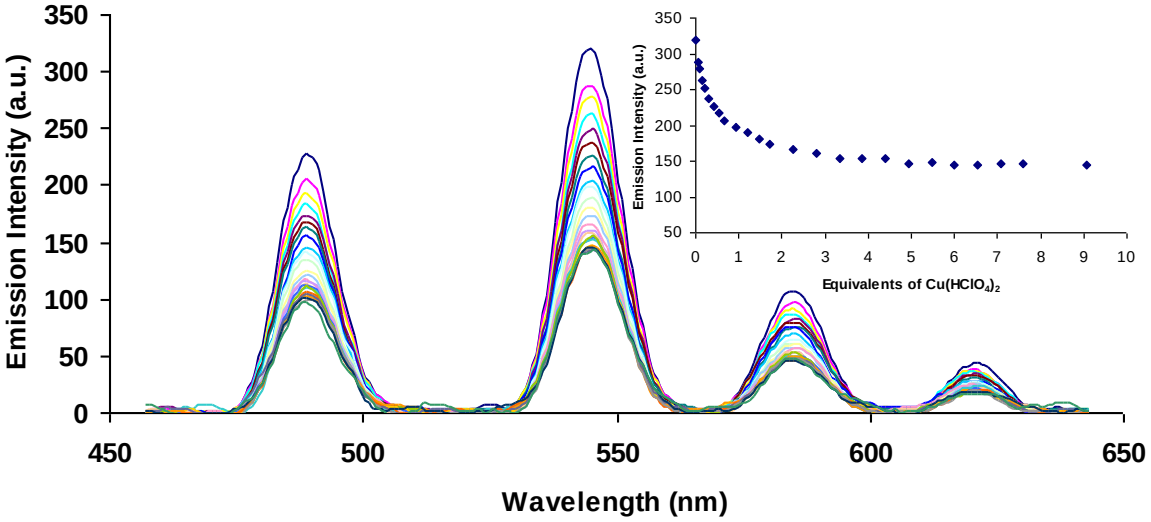


Figure S7d. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of $Cu(HClO_4)_2$ in H_2O. Inset: Plot of intensity at 545 nm with equivalents of $Cu(HClO_4)_2$ added.

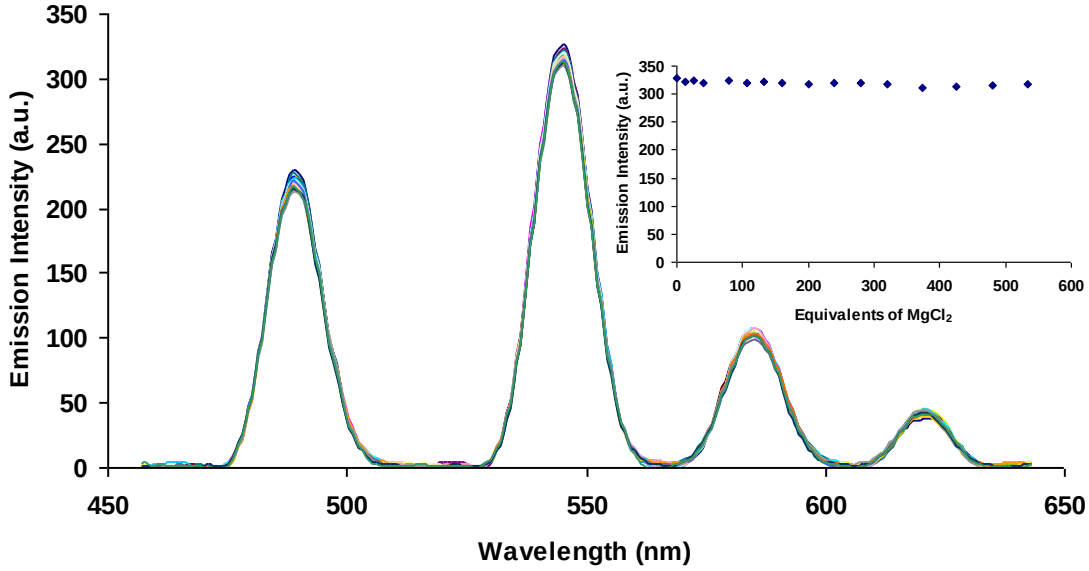


Figure S7e. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of $MgCl_2$ in H_2O. Inset: Plot of intensity at 545 nm with equivalents of $MgCl_2$ added.

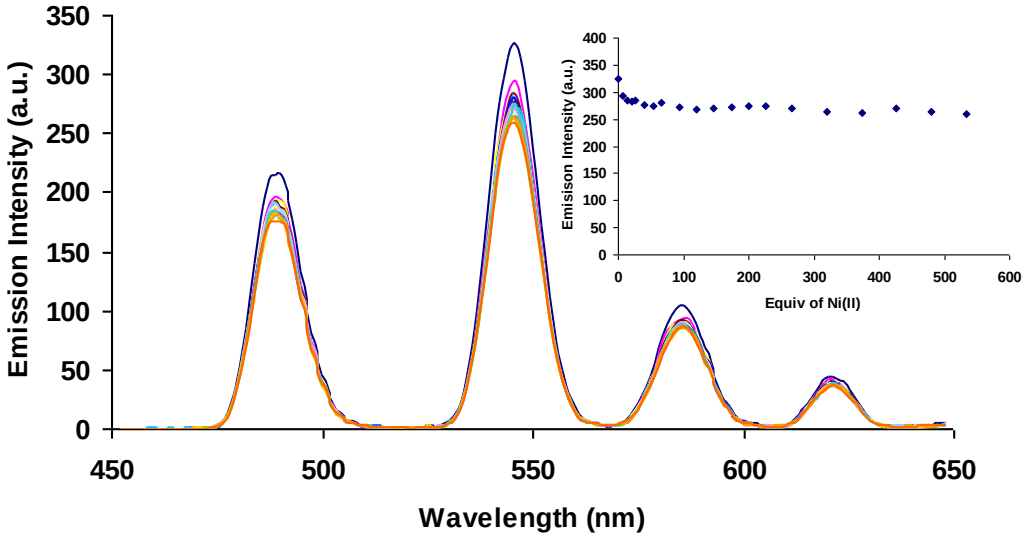


Figure S7f. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of $NiCl_2$ in H_2O. Inset: Plot of intensity at 545 nm with equivalents of $NiCl_2$ added.

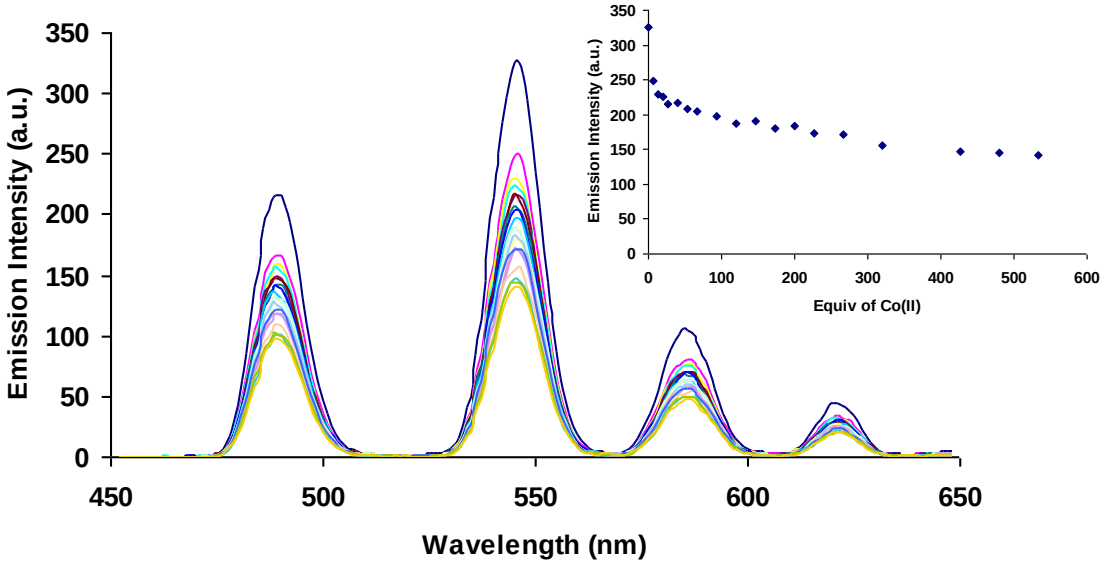


Figure S7g. Changes in the Tb^{III} emission of **1.Tb.Na** (10 μM) in the presence of $CoCl_2$ in H_2O. Inset: Plot of intensity at 545 nm with equivalents of $CoCl_2$ added.

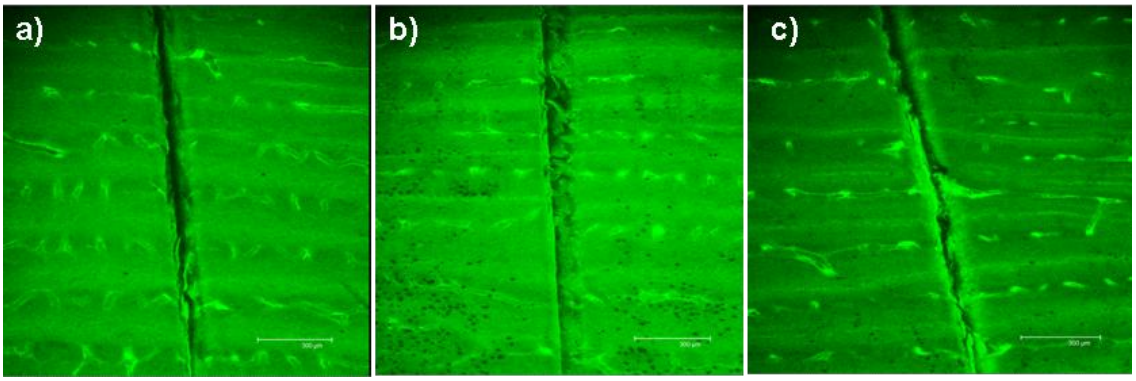


Figure S8a. Confocal laser-scanning microscopy images of bone sample immersed in a 1 x 10^{-3} M solution of **1.Tb** (20 mM HEPES, 135 mM KCl, pH 7.4) for different periods of time (a) Control, (b) 4 hr, (c) 24 hr; bar = 300 μm

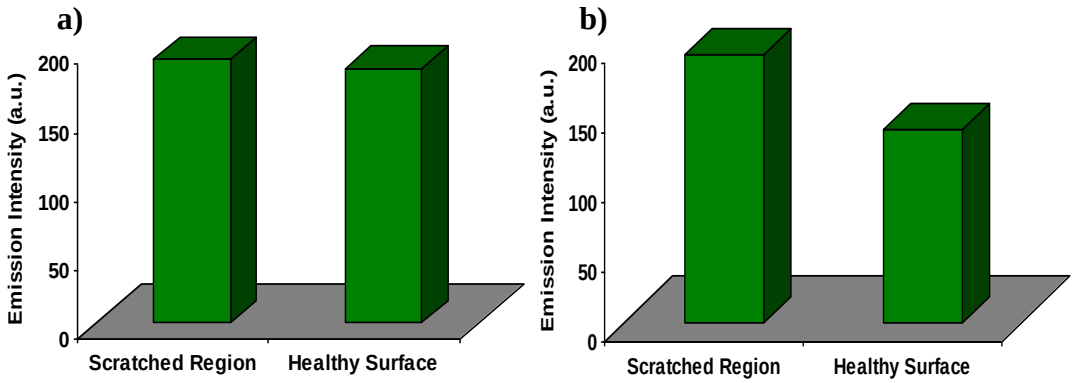


Figure S8b. Emission intensity enhancement between the scratched region and the outer healthy bone surface after a) 4 hr immersion and b) 24 hr immersion in a 1 x 10^{-3}M aqueous solution of **1.Tb**.

2. Protocol for Sectioning, Polishing, *in-vivo* Scratching and Staining of the bovine tibiae specimens.[1]

- Fresh bovine tibiae were obtained from a meat wholesalers, the soft tissue was removed and the bone stored at -20°C until required. Longitudinal sections of cortical bone from the mid-diaphysis were cut into beams (15 x 3 x 1 mm) using a low speed diamond saw and polished using grades of sand papers up to 1200 grit.
- A 5 mm line was scratched using a surgical scalpel on the surface of each beam and the specimens was immersed in a vial containing a 1 x 10^{-3}M solution of **1.Tb.Na** (20 mM HEPES, 135 mM KCl, pH 7.4) for 20 hours, the specimens were removed from the aqueous solution and a second 5 mm scratch was made.
- The samples were returned to the solution for a remaining four hours. After final removal of the bone specimens from the **1.Tb.Na** solution, a final 5 mm scratch was made. This will behave as the control, as this new scratch was never in contact with **1.Tb.Na**.
- Before being viewed using Confocal laser-scanning microscopy, the samples were washed with de-ionised water and mounted on a microscopy slide.
- Overall this procedure allowed the comparison of four different immersion times, 0 mins (control), 4 hrs and 24 hrs.

[1] (a) Parkesh, R.: Mohsin, S.; Lee, T.C.; Lee, Gunnlaugsson, T. *Chem. Mater*. **2007**, *19,* 1656.
(b) O' Brien, F. J.; Taylor, D.; Dickson, G. R.; Lee, T. C. *J. Anat*. **2000**, 197, 413.