Use of Yb(III) Centered Near Infra-Red (NIR) Luminescence to Determine the Hydration State of a 3,2-HOPO based MRI-Contrast Agent

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Supporting Information

Table of Contents

1. Detailed Synthesis of [Yb(Tren-3,2-HOPO)]

General

All solvents for reactions were used as supplied. The Tren-Me-3,2-HOPO ligand was prepared as previously described.[1] Elemental analyses were performed by the Microanalytical Laboratory, University of California, Berkeley, CA.

Complex Synthesis

39.8 mg (60.9 μmol) of Tren-3,2-HOPO·HCl·6H2O and 24.8 mg (64.0 μmol) of $YbCl_3 \cdot 6H_2O$ (99.99%) were suspended in 5 mL MeOH. The solution was heated to reflux temperature, followed by the addition of 50.0 μL of pyridine and then held at reflux temperature for *ca*. 14 hours. After cooling to room temperature, slow evaporation of the MeOH solvent at room temperature over a period of several days afforded a precipitate, which was collected by filtration and yielded 17.01 mg (16.1 μmol, 26.5 %) of the desired complex as a light beige solid. Elemental Analysis; Found (Calc'd) C, 32.00 (31.86); H, 4.73 (4.77); N, 9.35 (9.29) % for $[Yb(C_{27}H_{30}N_7O_9)]\cdot 4HCl\cdot 6H2O\cdot MeOH$

2. Details of Crystallographic Refinement

Crystals suitable for X-ray analysis were mounted on a Kapton loop using Paratone N hydrocarbon oil and measured at low temperature using a Siemens SMART CCD[2] area detector with graphite monochromated Mo-Kα radiation. Cell constants and orientation matrices were obtained from least-squares refinement. The resulting data were integrated by the program SAINT[3] and corrected for Lorentz and polarization effects. Data were also analyzed for agreement and possible absorption using XPREP.[4] An empirical absorption correction based on the comparison of redundant and equivalent reflections was applied using SADABS.[5] Equivalent reflections where appropriate were merged and no decay correction was applied. The structures were solved within the WinGX[6] package by direct methods using SIR92[7] and expanded using full-matrix least-squares techniques with SHELXL-97[8]. Hydrogen atoms were positioned geometrically, with C–H = 0.93 Å for CH aromatic, C–H = 0.97 Å for CH_2 methylene, N–H = 0.89 Å, and C–H = 0.96 Å for CH_3 methyl. Hydrogen atoms were constrained to ride on their parent atoms, with $U_{iso}(H)$ values set at 1.2 times $U_{eq}(C)$ for all H atoms. Resulting drawings of molecules were produced with Mercury-1.4.2.[9]

3. Attempted Competition Titration versus DTPA

Initial attempts to determine the solution stability of [Yb(Tren-Me-3,2-HOPO)(H_2O)$_2$] were performed by competition batch titration using DTPA as a known competitor, as detailed elsewhere.[10] Importantly, in the present instance, we chose to follow the decrease in percent speciation of the [Yb(Tren-3,2-HOPO)(H_2O)$_2$] complex upon DTPA addition via its (anticipated) decrease in NIR luminescence signal, since only this complex is luminescent, allowing for a more direct measure of the [Yb(Tren-3,2-HOPO)(H_2O)$_2$] concentration. Previously, evaluation of the complex concentration has been performed using absorbance measurements, which require deconvolution since both the 'complexed' and 'free' ligand contain a UV-absorbing chromophore.

However, as shown in Figure S1 below, we noted that instead of the expected decrease in luminescence signal due to competitive formation of the [Yb(DTPA)] complex, the Yb(III) centered emission ***increased*** until 1 molar equivalent of DTPA was added, then subsequently decreased. While unexpected, we can attribute this behavior to the formation of ternary [Yb(Tren-Me-3,2-HOPO)(DTPA)] complexes with either MLL′ or M_2L_2L' (*ie*. ML-L′-LM) stoichiometry, and hence the initial increase in luminescence can be attributed to the replacement of metal bound solvent water molecules in the first coordination sphere of the Yb(III), which have a known quenching effect.[11] Hence, stability measurements were instead performed by spectrophotometric pH titration, detailed in Section 4, Supp Info.

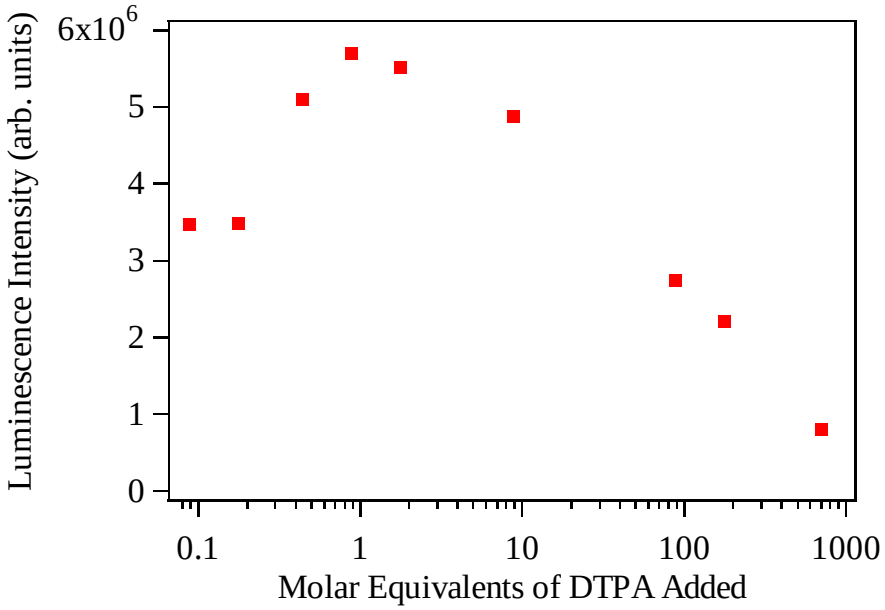

Fig S1. The observed luminescence intensity versus molar equivalent of added DTPA in aqueous TRIS buffer, pH 7.4 (with 0.1 M KCl)

4. Details of Solution Thermodynamic Experiments

The experimental protocols for spectrophotometric titrations closely followed those of a previous study.[12] Briefly, a titration vessel was charged with 100 mL (± 0.05 mL) of degassed 0.1 M KCl, and MES and HEPES as external non-coordinating buffers were added as concentrated solutions (*ca*. 0.2 M) in order to achieve final concentrations of *ca*. 0.5 mM to ensure proper pH buffering. After acquisition of a reference (blank) spectrum, addition of the ligand as a solid was executed to give a final concentration of approximately 25 μM. Subsequently, one equivalent of standardized Yb(III) was added *via* Eppendorf micropipette, and an aliquot of standardized 0.1 M HCl (200 μL) was added to adjust the pH to approximately 2.5. Solutions were titrated with 0.1 M KOH (using aliquots of *ca*. 0.5 – 1.0 mL) to an endpoint of pH 10.0, and subsequently back-titrated with standardized 0.1 M HCl (using aliquots of of *ca*. 0.5 – 1.0 mL) to pH 2.0, with equilibration times of 600 s between each addition. Absorbance spectra (250-500 nm, 2 nm data interval) were collected and the pH recorded for each addition. The data from each titration (absorbance *vs.* pH) were imported using pHab for data analysis and were treated by non-linear least squares refinement. All equilibrium constants are defined in terms of β_{mlh}, using the equation;

$$m\mathrm{M} + l\mathrm{L} + h\mathrm{H}^{+} \leftrightarrow \mathrm{M}_m\mathrm{L}_l\mathrm{H}_h \qquad\qquad \beta_{mlh} = \frac{[\mathrm{M}_m\mathrm{L}_l\mathrm{H}_h]}{[\mathrm{M}]^m[\mathrm{L}]^l[\mathrm{H}^+]^h}$$

Hydrolysis constants for Yb(III) ($\beta_{10\text{-}1}$ = -6.99, $\beta_{10\text{-}2}$ = -14.84, $\beta_{10\text{-}3}$ = -23.14, $\beta_{10\text{-}4}$ = -32.39) were estimated using the methods described in Baes and Mesmer[13] for an ionic strength of I = 0.1 M. These were included as fixed values in the refinement, together with the values for ligand proton association constants obtained by potentiometry (β_{011} = 8.2, β_{012} = 15.15, β_{013} = 20.95, β_{014} = 25.91) as reported elsewhere.[1] Absorbances for colored species were refined only when present above 5% under the experimental conditions, and the species ML, MLH, L, LH, LH_2 and LH_3 were defined as absorbing. All experiments were performed at 25°C and with 0.1 M KCl as electrolyte. Each determination results from three independent experiments, where each experiment consists of two titrations, first against 0.1 M HCl followed by reverse titrations against 0.1 M KOH.

5. Details of Photophysical Experiments

UV-Visible absorption spectra were recorded on Varian Cary 300 double beam absorption spectrometer using quartz cells of 1.0 cm path length. Steady state emission spectra were acquired on a HORIBA Jobin Yvon IBH FluoroLog-3 (FL-3) spectrofluorimeter. The excitation light source was a 450 W Xe arc lamp and spectral selection was achieved by passage through a double grating excitation monochromator (1200 grooves/mm) blazed at 330 nm. Spectra were reference corrected for the excitation light source variation (lamp and grating). Emission spectra were collected at 90° to the excitation, and emission was collected on the 'T' side using a Hamamatsu H9170-75 NIR as the detector. Spectral selection was achieved by passage through a double grating emission monochromator (600 grooves/mm) blazed at 1 μm. The emission spectral response of the Hamamatsu detector is essentially linear across the 950-1700 nm wavelength range, and, since the Yb(III) signals are narrow (*ca*. 60 nm at FWHM), the observed emission was not corrected for the efficiency of the grating.

Luminescence lifetimes were determined with a HORIBA Jobin Yvon IBH FluoroLog-3 spectrofluorimeter, adapted for time resolved measurements. An N_2 laser (LN1000, Laser Photonics, Inc) λ_{ex} = 337.1 nm was used as the light source, coupled to the entrance port of the FluoroLog-3. The input pulse energy was *ca*. 1.5 mJ/pulse, with an optical pulse duration of less than 800 ps FWHM. A portion of this excitation was sampled with a quartz beam sampling plate, the specular reflection of which was focused onto the entrance of a UV-sensitive photodiode (DET210, Thor Labs). The small amplitude analogue output from the photodiode was processed into a TAC Start signal (NIM) using a TB-01 pulse converter module from IBH. A Hamamatsu H9170-75 NIR fast rise time PMT operating at -800 V and -60°C was used as the detector, and the output signal from the PMT was processed using a TB-02 0.5 GHz pre-amplifier module from IBH, and a 100 MHz Constant Fraction Discriminator (CFD) (Model 6915, Phillips Scientific), yielding appropriate TAC Stop signals (NIM). These were acquired using a 2 ns PCI Multi Channel Scaling (MCS) card (Model P7888-1E, FAST ComTec GmbH), and processed using the DataStation software package from IBH. Data analysis was performed using the commercially available DAS 6 decay analysis software package from HORIBA Jobin Yvon IBH. Goodness of fit was assessed by minimizing the reduced chi squared function, χ^2, and a visual inspection of the weighted residuals. Each trace contained at least 5,000 points and the reported lifetime values result from three independent measurements. The estimated error on these values is ± 10 %. Typical sample concentrations for absorption and fluorescence measurements were *ca*. 10^{-5} M and 1.0 cm cells in quartz suprasil or equivalent were used.

6. Sample of Yb(III) lifetime data in H_2O and D2O

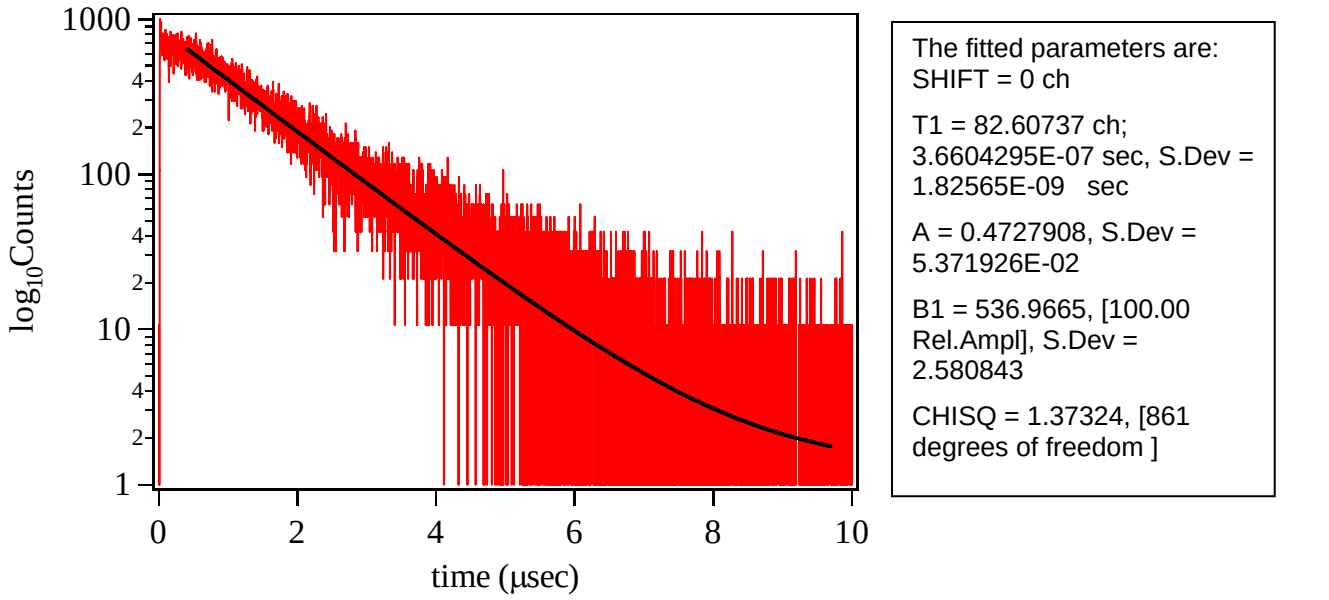

Fig S2. The observed MCS Decay (red) and monoexponential fit (black) together with fitting parameters for [Yb(Tren-Me-3,2-HOPO)()] in 0.1 M TRIS buffer (pH 7.4).

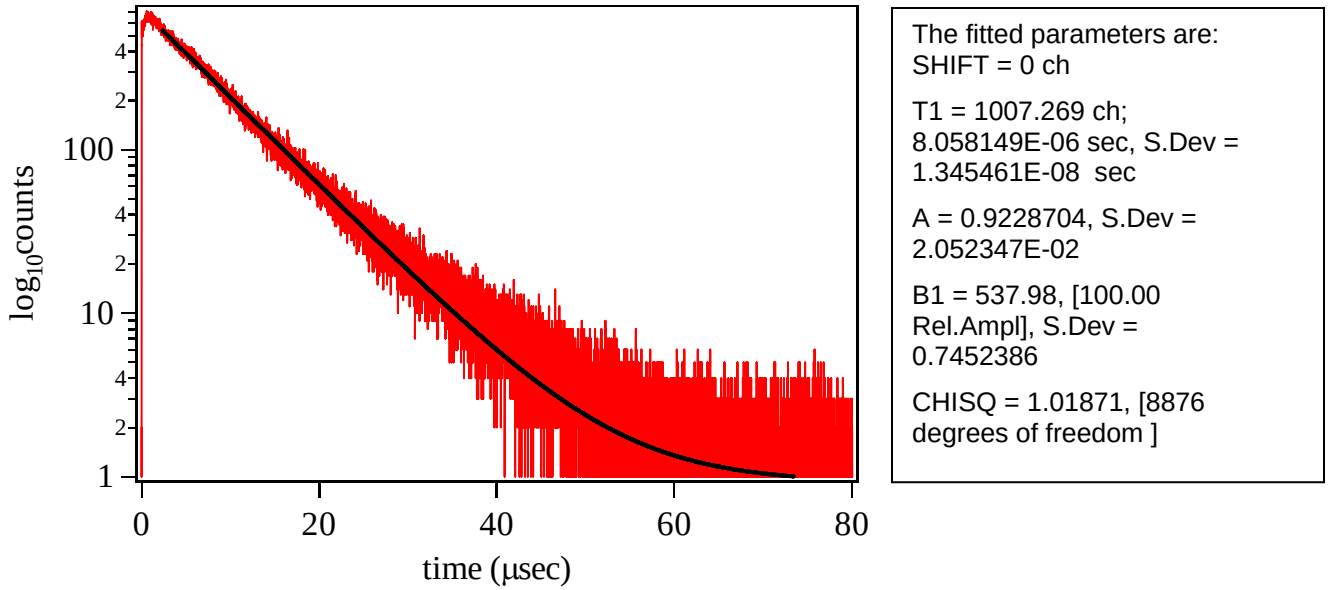

Fig S3. The observed MCS Decay (red) and monoexponential fit (black) together with fitting parameters for [Yb(Tren-Me-3,2-HOPO)()] in D_2O (*ca*. pH 6).

7. References

1. Xu, J.; Franklin, S. J.; Whisenhunt, D. W., Jr.; Raymond, K. N., *J. Amer. Chem. Soc.*, **1995**, *117*, 7245-6.
2. *SMART, Version 5.059: Area-Detector Software Package; Bruker Analytical X-ray Systems, Inc.: Madison, WI,* **1999**.
3. *SAINT, Version 7.07B: SAX Area-Detector Integration Program; Siemens Industrial Automation, Inc.: Madison, WI,* **2005**.
4. *XPREP, Version 6.12: Part of the SHELXTL Crystal Structure Determination Package; Bruker AXS Inc.: Madison, WI,* **1995**.
5. Sheldrick, G. *SADABS, Version 2.10: Siemens Area Detector Absorption Correction Program; University of Göttingen: Göttingen, Germany,* **2005**.
6. *WinGX 1.70.01: Farrugia, L. J. J. Appl. Crystallogr.* **1999**, *32, 837-838.*
7. *SIR92: Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. J. Appl. Crystallogr.* **1993**, *26, 343-350.*
8. *Sheldrick, G. M. SHELX97 - Programs for Crystal Structure Analysis; Institut fur Anorganische Chemie der Universitat:Gottingen, Germany*, **1998**.
9. *Mercury: Visualization and Analysis of Crystal Structures.* C. F. Macrae, P. R. Edgington, P. McCabe, E. Pidcock, G. P. Shields, R. Taylor, M. Towler and J. van de Streek, *J. Appl. Cryst.*, **2006,** *39*, 453-457.
10. Pierre, Valerie C.; Botta, Mauro; Aime, Silvio; Raymond, Kenneth N. Inorg. Chem., **2006**, *45*, 8355-8364.
11. Faulkner, S; Beeby, A.; Dickins, R. S.; Parker, D.; Williams, J. A. G., *J. Fluorescence.*, **1999**, 9, 45-49.
12. Xu, J.; O'Sullivan, B.; Raymond, K. N., *Inorg. Chem.*, **2002**, *41*, 6731-6742.
13. Baes, C. F.; Mesmer, R. E. *The Hydrolysis of Cations*; Wiley-Interscience: New York, **1976**.