Supporting Information

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I. CEST Spectroscopy

CEST spectra of 20 mM solutions of Tb•2 in 50% water in CD_3CN were recorded on a Varian INOVA 400 spectrometer. NMR samples containing different pH values ranging from 5.0 to 8.0 were trapped in 5 mm sample tubes and scanned at 298 and 310 K, respectively. CEST spectra were generated by plotting the residual bulk water signal intensity ratio as a function of saturation frequency. A pre-saturation pulse of 3 s duration was applied at 498 Hz. The pH values of the samples were measured using a Corning 125 pH meter with a calibrated combined microelectrode purchased from Aldrich Chemical Co.

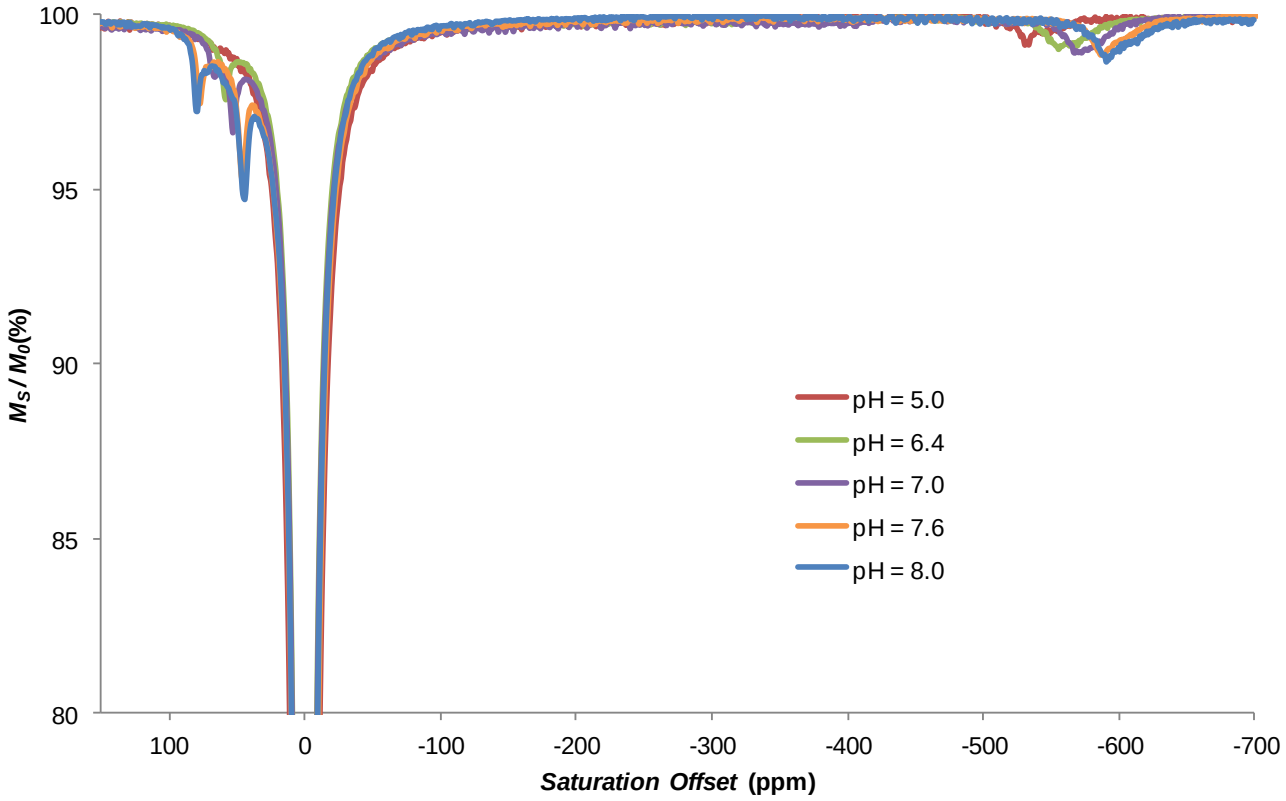


Figure S1a. CEST spectra of 20mM Tb•2 agent recorded at 298 K with B_1 of 11.7 μT.

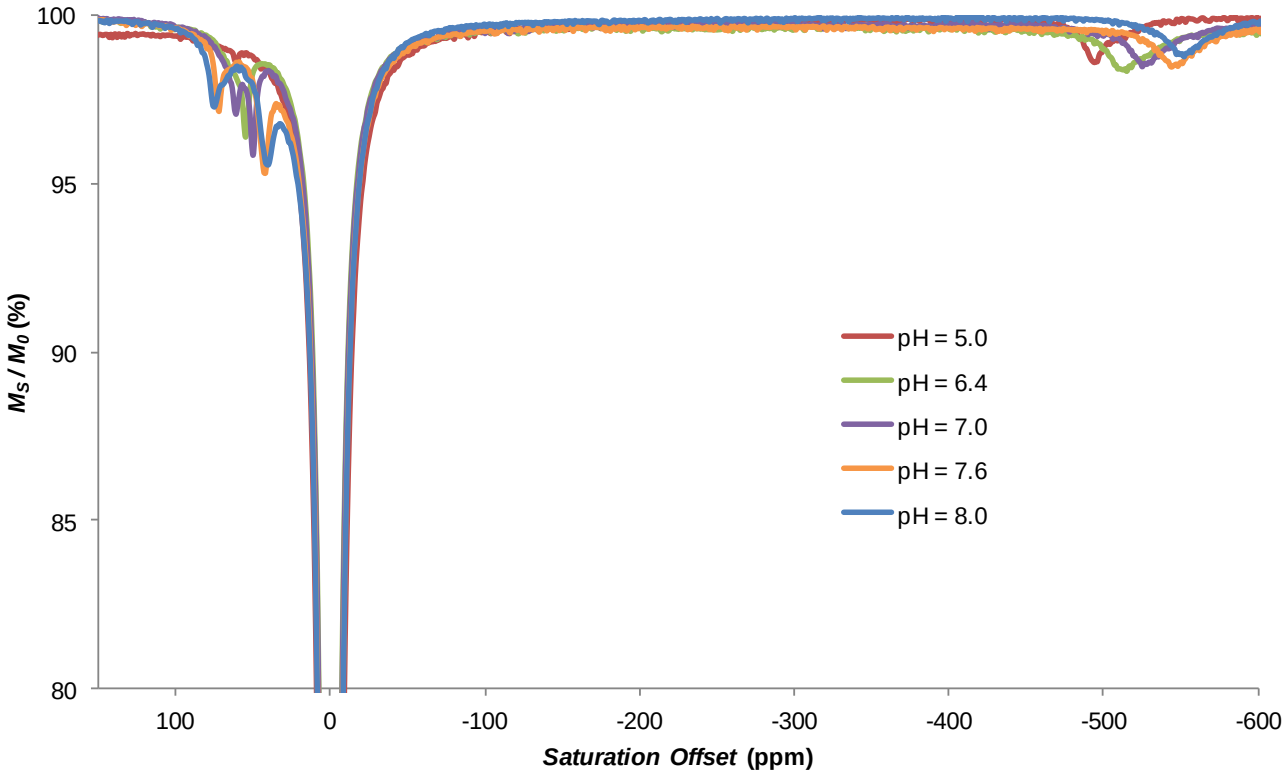


Figure S1b. CEST spectra of 20mM Tb•2 agent recorded at 310 K with B_1 of 11.7 μT.

Two samples containing 20mM Tb•2 agents at pH 7 were prepared. One contained just the solution, whereas the other contained the solution mixed with minced mouse kidney tissue to generate MT effects similar to those observed in biological tissue. Asymmetry analysis is performed with respect to the free water frequency assigned to 0 ppm.

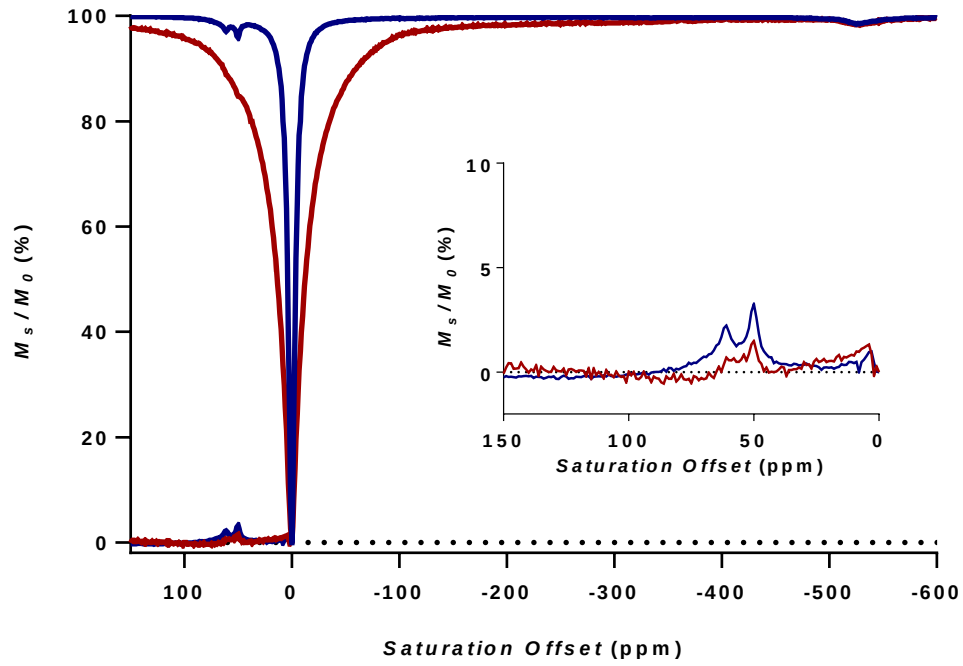


Figure S2. CEST spectra of 20mM Tb•2 agent recorded at 310K and pH 7.0 in CD_3CN/H_2O (1:1) with B_1 of 11.7 μT. Tb•2 agent only: blue line; Tb•2 agent mixed with minced kidney tissue of mice: red line. The inset enlarges the size of CEST asymmetry spectra.

II. Measurement of the Bound Water Lifetime (τ_B) by T_{2exch}

For *in vitro* data collection, five different concentrations of Tb•2 were prepared (1, 3, 6, 8, and 12 mM) at pH 5.0 and (1.25, 2.5, 4, 5 and 8 mM) at pH 8.0, respectively. The total T_2 (T_{2tot}) of each sample was measured using a Maran Ultra 0.54 T spectrometer with three different temperatures controlled at 25, 37 and 52 ^{o}C. The Carr–Purcell–Meiboom–Gill sequence and a nonlinear least-squares fitting procedure were used to process the data. Linear fits were used to calculate the total transverse relaxivity (r_{2tot}) for each temperature and the slope of each linear fit gives the value of r_{2tot} for the agent. All the samples were allowed to equilibrate in the probe for 15 minutes at each temperature before data acquisition.

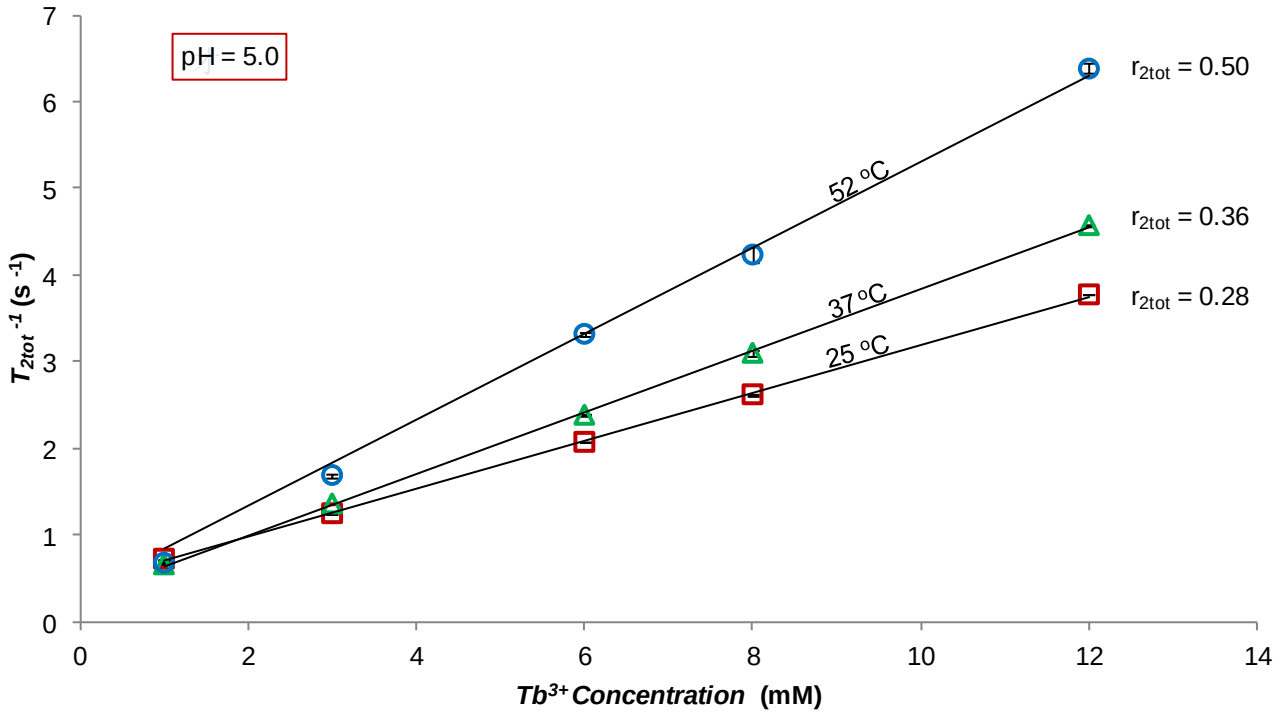


Figure S3. A plot of total transverse relaxation rate (T_{2tot}^{-1}) versus Tb^{3+} concentration (mM) for Tb•2 at 0.54 T (23 MHz) and 25, 37 and 52 ^{o}C, respectively. The pH values of the samples are 5.0. The r_{2tot} values for each linear fit are given in mM^{-1} s^{-1} and directly proportional to temperature.

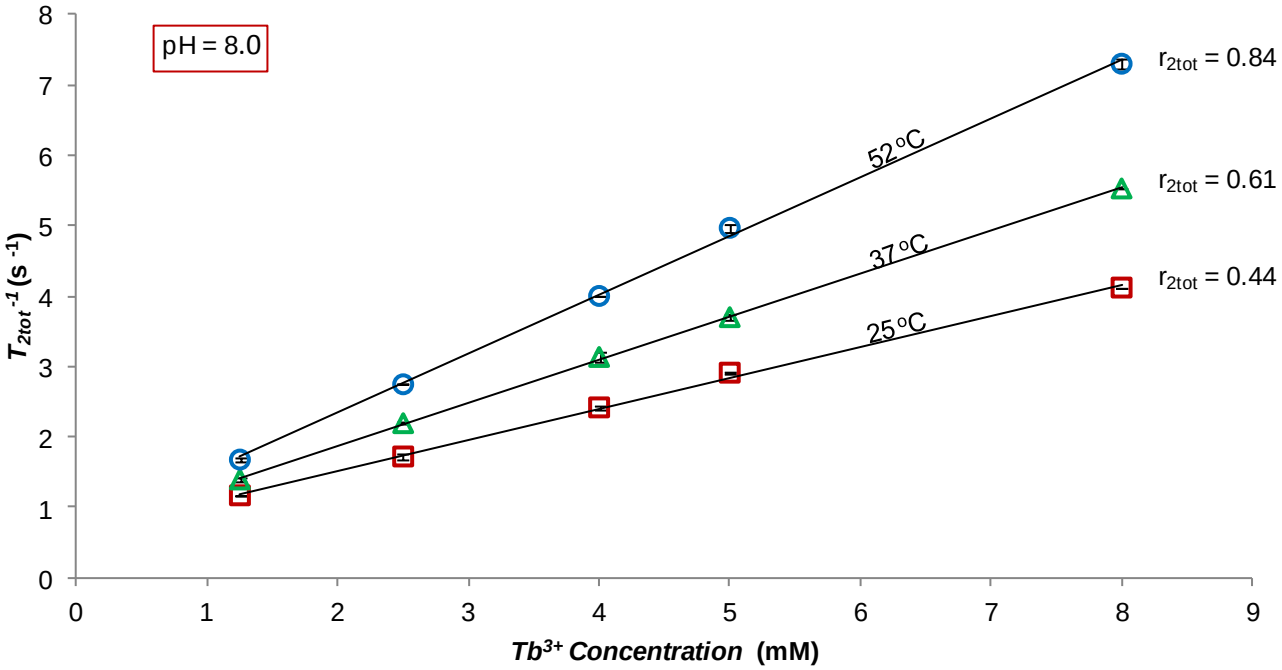


Figure S4. A plot of total transverse relaxation rate (T_{2tot}^{-1}) versus Tb^{3+} concentration (mM) for Tb•2 at 0.54 T (23 MHz) and 25, 37 and 52 °C, respectively. The pH values of the samples are 8.0. The r_{2tot} values for each linear fit are given in mM^{-1} s^{-1} and directly proportional to temperature.

The transverse relaxivity due to water molecule exchange (r_{2exch}) was then calculated by subtracting the total transverse relaxivity of TbTETA from r_{2tot} (r_{2exch} = r_{2tot} – $r_{2TbTETA}$). Note that for TbTETA, which has no water molecule exchange, its r_{2tot} is relatively independent of temperature. The measurement error in r_{2tot} from the least squares fitting was less than 10% and the Tb^{3+} concentration of each sample was determined by ICP-OES analysis.

Table S1
Measured r_{2t0t}, r_{2exch} and τ_B values for Tb•2 taken at 298 and 310K, respectively, where r_{2exch} = r_{2tot} – $r_{2TbTETA}$.

Tb^{3+} chelate	Total transverse relaxivity r_{2tot} (mM^{-1} s^{-1})				Exchange transverse relaxivity r_{2exch} (mM^{-1} s^{-1})				Bound water molecule lifetime τ_B (μs)			
	25 °C		37 °C		25 °C		37 °C		25 °C		37 °C	
	pH=5	pH=8	pH=5	pH=8	pH=5	pH=8	pH=5	pH=8	pH=5	pH=8	pH=5	pH=8
Tb•2	0.28	0.44	0.36	0.61	0.16	0.36	0.24	0.53	224	149	106	66
TbTETA	0.12	0.08	0.12	0.08								

Swift-Connick theory[1] predicts that the transverse relaxivity due to water molecule exchange (r_{2exch}) is a function of the bound water molecule lifetime (τ_B) as given by :

$$r_{2exch} = (3.6 \times 10^{-5}) \frac{\tau_B \Delta\omega^2}{1+\tau_B{}^2 \Delta\omega^2} \quad [1]$$

where Δω is the paramagnetic frequency shift of the bound water molecule protons expressed in rad s^{-1}. The Plots of r_{2exch} versus τ_B, hereafter called a ''Swift–Connick'' plot for Tb^{3+} at 0.54 T at pH 5.0 (Δω = -534 ppm or 7.72 × 10^4 rad s^{-1} at 25 °C and Δω = -494 ppm or 7.14 × 10^4 rad s^{-1} at 37 °C) are shown in Figures S5 and S6. Swift-Connick Plots of r_{2exch} versus τ_B for Tb^{3+} at 0.54 T at pH 8.0 (Δω = -591 ppm or 8.54 × 10^4 rad s^{-1} at 25 °C and Δω = -550 ppm or 7.95 × 10^4 rad s^{-1} at 37 °C) are shown in Figures S7 and S8. Equation [1] predicts that for Tb^{3+} at pH 5.0 and 25 °C, the r_{2exch} reaches a peak value of 1.38 mM^{-1} s^{-1} at a specific bound water lifetime τ_B of 13 ns. Accordingly the "fast" side of the Swift-Connick curve is defined as τ_B < 13 ns, while the "slow" side is defined as τ_B > 13 ns. The fast and slow exchange at different pH and temperature is defined in similar manner.

The previously calculated exchange transverse relaxivities (summarized in Table S1) were used to determine the bound water lifetimes in Figures S5-S8).

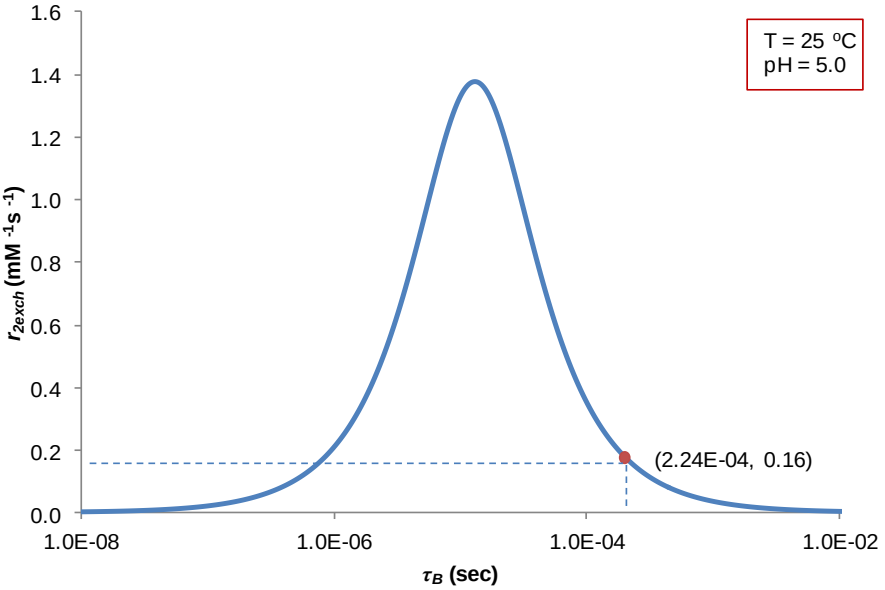


Figure S5. A Swift–Connick plot of transverse relaxivity r_{2exch} (mM^{-1} s^{-1}) versus bound water lifetime τ_B (s) for Tb^{3+} at 0.54 T and 25 °C. A Δω of -534 ppm was used to plot the curve.

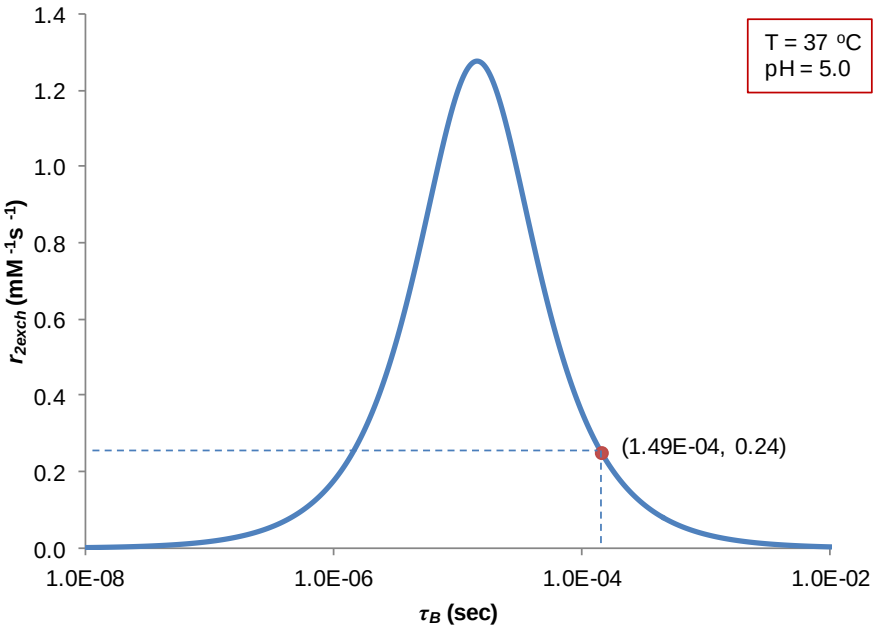


Figure S6. A Swift–Connick plot of transverse relaxivity r_{2exch} (mM^{-1} s^{-1}) versus bound water lifetime τ_B (s) for Tb^{3+} at 0.54 T and 37 °C. A $\Delta\omega$ of -494 ppm was used to plot the curve.

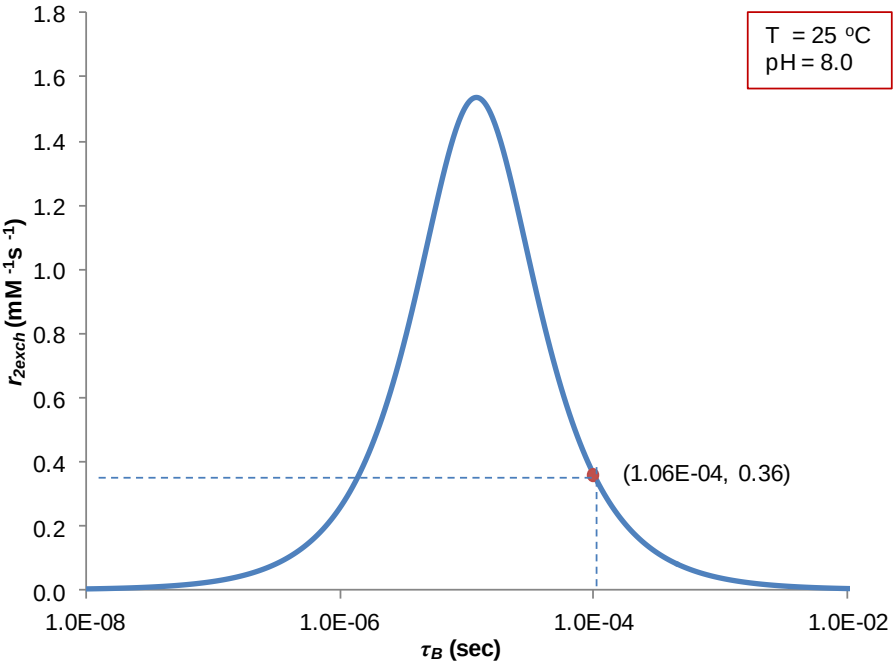


Figure S7. A Swift–Connick plot of transverse relaxivity r_{2exch} (mM^{-1} s^{-1}) versus bound water lifetime τ_B (s) for Tb^{3+} at 0.54 T and 25 °C. A $\Delta\omega$ of -591 ppm was used to plot the curve.

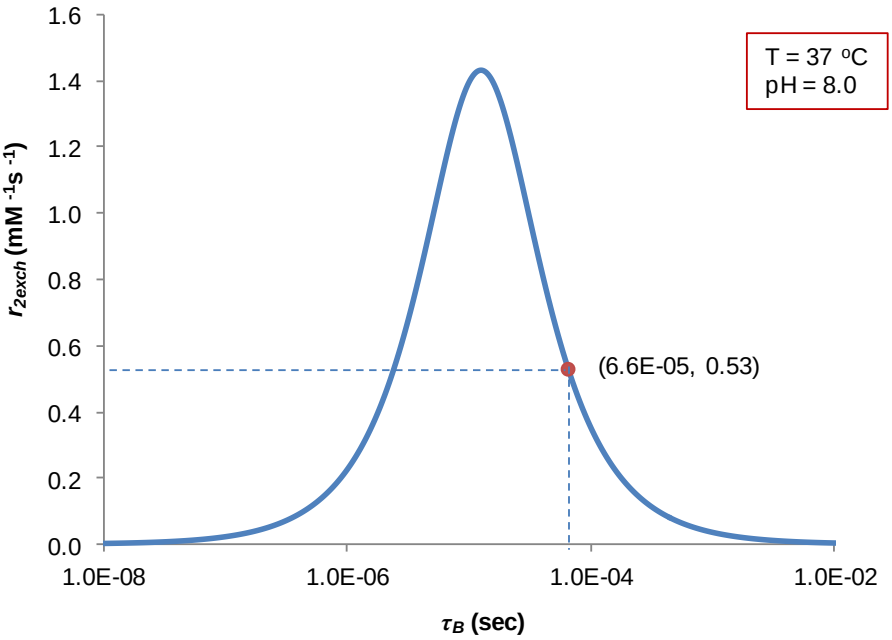


Figure S8. A Swift–Connick plot of transverse relaxivity r_{2exch} (mM^{-1} s^{-1}) versus bound water lifetime τ_B (s) for Tb^{3+} at 0.54 T and 37 °C. A $\Delta\omega$ of -550 ppm was used to plot the curve.

III. Imaging Protocols

A phantom of seven samples (20 mM) in bundled capillaries was used for phantom MR imaging. The sample pH values were maintained in the pH range from 5.5 to 8.2 using MES or HEPES buffers (5 mM). The sample temperature was regulated at 37.0 ± 0.1 °C with an animal monitoring system from Small Animal Instruments (Stony Brook, NY). The phantom image were acquired with an Agilent 9.4 T (400 MHz) small animal MR imaging system equipped with a homemade surface nano-coil. The MRI data were acquired using a steady state gradient echo pulse sequence (GEMS) with centric K-space encoding preceded by a rectangular saturation pulse (power = 100 μT and duration = 5 s). The pre-saturation frequency offset was varied from 100 to -800 ppm with a frequency offset step of 5 ppm. Other acquisition parameters are TR = 5.2 s, TE = 2.9 ms, average = 1, FOV = 4 mm × 4 mm, matrix = 32 × 32, and flip angle = 30°.

IV. Imaging Processing

The CEST images were processed using self-written scripts in MATLAB R2009a (The Mathworks, Inc., Natick, MA, USA). The image data were analyzed at a voxel-by-voxel level. Groups of 3 × 3 adjacent voxels were binned to improve the signal-to-noise ratio of the center voxel. The Z spectra arising from the image voxels were fitted to Lorentzian line shapes. The noise filter (CEST effect < 1%) was applied to remove the CEST signals arising from the noisy data. Finally, according to the pH calibration curve (Figure 1 inset), the pH maps were converted from the CEST peak frequency maps obtained from the fitted Z spectra.

V. Experimental

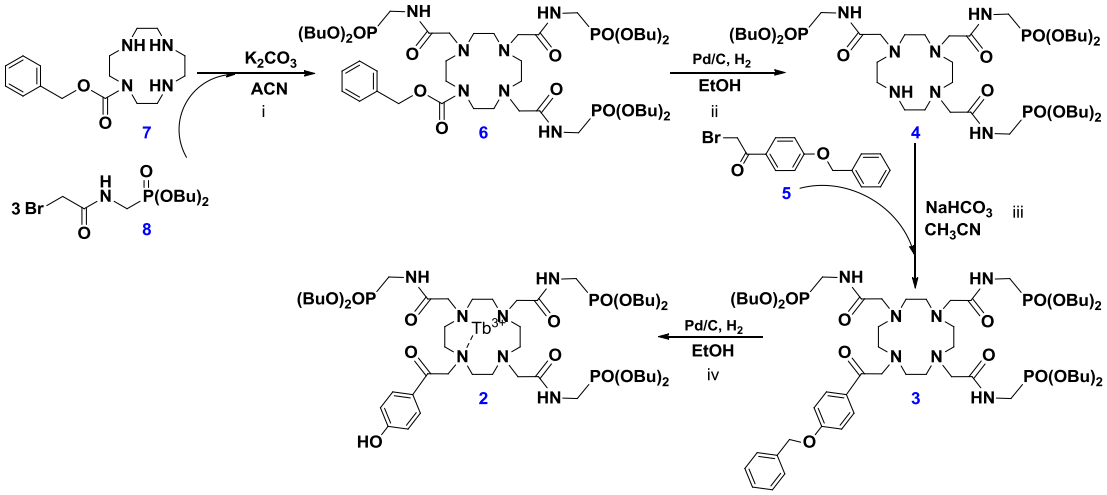
General Remarks

All reagents and solvents were purchased from commercial sources and used as received unless otherwise stated. ^{1}H and ^{13}C NMR and CEST spectra were recorded on a Varian INOVA 400 spectrometer. The chemical shift values were reported relative to TMS and given in ppm (δ-scale). CEST images were recorded on an Agilent 9.4 T small animal imaging system. The pH values of the samples were measured at ambient temperature using a Corning 125 pH meter with a calibrated combined microelectrode purchased from Aldrich Chemical Co. The NMR sample was allowed to equilibrate in the probe for 10 minutes at each temperature before data acquisition. "Water" refers to high purity water with a resistance of >18 MΩ. ICP analyses were done by the Galbraith Laboratories, Inc (Knoxville, TN). ESI LC-MS spectra were recorded with a Waters QtofMS-XEVO instrument.

Synthesis of Ligand 2

The ligand was synthesized according to Scheme S1. Mono-Cbz protected cyclen **7**, bromo-methyl-acetamide-methylene phosphonate **8** and bromo-methyl-phenol **5** were synthesized according to literature procedures.[2]

Scheme S1. Synthesis of Ligand 2



1-Benzyloxycarbonyl-4,7,10-tris-(aminomethylphosphonic acid dibutyl ester) amide-1,4,7,10-tetraazacyclododecane (6). A mixture of mono-Cbz protected cyclen **7** (3.91 g, 12.76 mmol), dibutyl (2-bromoacetamido)methylphosphonate **8** (13.63 g, 39.60 mmol) and potassium carbonate (5.53 g, 39.60 mmol) in acetonitrile was heated at 55 ^{o}C for 3 days. The mixture of solution was cooled down to room temperature and poured through a filter paper in a Buchner funnel. The filtrate was evaporated under vacuum and purified by column chromatography on silica gel, eluting with 5:95 methanol-chloroform to yield 12.30 g (11.22 mmol, 88%) of sticky yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (s, 2H, Ph), 7.23 (m, 3H, Ph), 5.00 (s, 2H, PhC$\underline{H}_2$O), 3.94 (q, 12H, POC$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 3.62 (dd, 4H, NHC$\underline{H}_2$PO), 3.43 (dd, 2H, NHC$\underline{H}_2$PO), 3.13 (s, 4H, N_{ring}C$\underline{H}_2$CO), 3.07 (s, 2H, N_{ring}C$\underline{H}_2$CO), 2.93 (b, 16H, N_{ring}C$\underline{H}_2$), 1.53 (m, 12H, POCH$_2$C$\underline{H}_2$CH$_2$CH$_3$), 1.28 (m, 12H, POCH$_2$CH$_2$C$\underline{H}_2$CH$_3$), 0.82 (t, 18H, $^{3}J_{H\text{-}H}$ = 7 Hz , POCH$_2$CH$_2$CH$_2$C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$) δ 170.8 (N_{ring}CH$_2$$\underline{C}$=O), 170.7 ($N_{ring}CH_2$$\underline{C}$=O), 156.2($N_{ring}$C=O), 136.6 (1-Ph), 128.5 (2-Ph), 128.0 (4-Ph), 127.8 (3-Ph), 67.1(Ph$\underline{C}$H$_2$O), 66.1(PO$\underline{C}$H$_2$CH$_2$CH$_2$CH$_3$), 66.0 ($N_{ring}$$\underline{C}H_2$CO), 54.5 ($N_{ring}$$\underline{C}H_2$), 54.0 ($N_{ring}$$\underline{C}H_2$), 53.3 ($N_{ring}$$\underline{C}H_2$), 52.0 ($N_{ring}$$\underline{C}H_2$), 34.2 (d, N$\underline{C}H_2$P, J_{CP} = 157 Hz), 32.4 (POCH$_2$$\underline{C}H_2CH_2CH_3$), 18.6 (POCH$_2CH_2$$\underline{C}H_2CH_3$), 13.5 (POCH$_2CH_2CH_2$$\underline{C}H_3$). *m/z* (ESMS ESI+): 1096 $[M+H]^+$, an appropriate isotope pattern was observed.

1,4,7-tris-(aminomethylphosphonic acid dibutyl ester) amide-1,4,7,10-tetraazacyclododecane (4). Compound **6** (8.19 g, 7.47 mmol) was dissolved in ethanol (50 mL). 10% palladium on carbon (0.8 g) was then added. The reaction mixture was shaken on a Parr hydrogenator under a H_2 pressure of 50 psi at room temperature for 18 hours. The mixture was filtered and the solvent was removed under reduced pressure to afford 6.91 g (7.18 mmol, 96%) of pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.00 (q, 12H, POC$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 3.65 (dd, 4H, NHC$\underline{H}_2$PO), 3.45 (dd, 2H, NHC$\underline{H}_2$PO), 3.25 (s, 4H, N_{ring}C$\underline{H}_2$CO), 2.97 (s, 2H, N_{ring}C$\underline{H}_2$CO), 2.64 (b, 16H, N_{ring}C$\underline{H}_2$), 1.57 (m, 12H, POCH$_2$C$\underline{H}_2$CH$_2$CH$_3$), 1.32 (m, 12H, POCH$_2$CH$_2$C$\underline{H}_2$CH$_3$), 0.86 (t, 18H, $^{3}J_{H\text{-}H}$ = 7 Hz , POCH$_2$CH$_2$CH$_2$C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$) δ 171.8 (N_{ring}CH$_2$$\underline{C}$=O), 171.6 ($N_{ring}CH_2$$\underline{C}$=O), 68.3(PO$\underline{C}H_2CH_2CH_2CH_3$), 68.2 ($N_{ring}$$\underline{C}H_2$CO), 56.8 ($N_{ring}$$\underline{C}H_2$), 56.4($N_{ring}$$\underline{C}H_2$), 54.3($N_{ring}$$\underline{C}H_2$), 52.6 ($N_{ring}$$\underline{C}H_2$), 35.5 (d, N$\underline{C}H_2$P, J_{CP} = 157 Hz), 33.1 (POCH$_2$$\underline{C}H_2CH_2CH_3$), 19.1 (POCH$_2CH_2$$\underline{C}H_2CH_3$), 13.9 (POCH$_2CH_2CH_2$$\underline{C}H_3$). *m/z* (ESMS ESI+): 962 $[M+H]^+$, an appropriate isotope pattern was observed.

1-[2-(4-hydroxyphenyl)-2-oxoethyl]-4,7,10-tris-(aminomethylphosphonic acid dibutyl ester) amide-1,4,7,10-tetraazacyclododecane (3). Compound **4** (6.56 g, 6.81 mmol), 2-bromo-1-[4-(phenylmethoxy)phenyl]-ethanone **5** (2.34 g, 7.50 mmol), and $NaHCO_3$ (0.64 g, 7.50 mmol) were added into acetonatrile (200 mL). The reaction mixture was heated at 65 ^{o}C under N_2 for 24 hours with stirring. The mixture was cooled to room temperature and filtered. The filtrate was evaporated to give

crude product. Then it was purified by column chromatography on silica gel, eluting with 20:80 methanol-chloroform to afford 6.88 g (5.80 mmol, 85%) of red brown oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (2H, d, $^{3}J_{H\text{-}H}$ = 8 Hz, Ph), 7.24-7.17 (5H, m, Ph), 6.81 (2H, d, $^{3}J_{H\text{-}H}$ = 8 Hz, Ph), 5.24 (s, 2H, PhC$\underline{H}_2$), 3.91 (m, 12H, POC$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 3.68 (dd, 2H, NHC$\underline{H}_2$P), 3.56 (dd, 4H, NHC$\underline{H}_2$P), 3.24 (s, 2H, N_{ring}C$\underline{H}_2$CON), 3.21(s, 6H, N_{ring}C$\underline{H}_2$CON), 2.83-1.99 (b, 16H, N_{ring}C$\underline{H}_2$), 1.46 (m, 12H, POCH$_2$C$\underline{H}_2$CH$_2$CH$_3$), 1.22 (m, 12H, $^{3}J_{H\text{-}H}$ = 7 Hz, POCH$_2$CH$_2$C$\underline{H}_2$CH$_3$), 0.76 (m, 18H, POCH$_2$CH$_2$CH$_2$C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$) δ 196.6 (C=O), 172.8 (C=O), 162.9 (Ph), 135.6 (Ph), 129.9 (Ph), 129.7 (Ph), 128.2 (Ph), 127.9 (Ph), 127.0 (Ph), 114.6 (Ph), 69.8 (Ph$\underline{C}$H$_2$), 65.9 (PO$\underline{C}$H$_2$CH$_2$CH$_2$CH$_3$), 61.4 ($N_{ring}\underline{C}$H$_2$CON), 59.2 ($N_{ring}\underline{C}$H$_2$CON), 50.5 (b, $N_{ring}\underline{C}$H$_2$), 34.3 (d, N$\underline{C}$H$_2$P, J_{CP} = 157 Hz), 32.2 (POCH$_2\underline{C}$H$_2$CH$_2$CH$_3$), 18.3 (POCH$_2$CH$_2\underline{C}$H$_2$CH$_3$), 13.3 (POCH$_2$CH$_2$CH$_2\underline{C}$H$_3$). *m/z* (ESMS ESI+): 1186 $[M+H]^+$, an appropriate isotope pattern was observed.

1-*N*-[2-[4-hydroxyphenyl]-2-oxoethyl]-4,7,10-tris-(aminomethylphosphonic acid dibutyl ester) amide-1,4,7,10-tetraazacyclododecane (2). Compound 3 (5.96 g, 5 mmol) was dissolved in ethanol (50 mL). 10% palladium on carbon (0.5 g) was then added. The reaction mixture was shaken on a Parr hydrogenator under a H_2 pressure of 50 psi at room temperature for 2 days. The mixture was filtered and the solvent was removed under reduced pressure to afford 5.21 g (4.75 mmol, 95%) of a hydroscopic solid. Melting point and combustion analysis could not be determined due to its hygroscopic nature. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, 2H, $^{3}J_{H\text{-}H}$ = 8 Hz , Ph), 6.88 (d, 2H, $^{3}J_{H\text{-}H}$ = 8 Hz, Ph), 3.92 (q, 12H, POC$\underline{H}_2$CH$_2$CH$_2$CH$_3$), 3.52 (dd, 6H, NHC$\underline{H}_2$P), 3.22 (b, 8H, N_{ring}C$\underline{H}_2$CON), 2.88-2.08 (b, 16H, N_{ring}C$\underline{H}_2$), 1.50 (m, 12H, POCH$_2$C$\underline{H}_2$CH$_2$CH$_3$), 1.25 (m, 12H, POCH$_2$CH$_2$C$\underline{H}_2$CH$_3$), 0.79 (m, 18H, POCH$_2$CH$_2$CH$_2$C$\underline{H}_3$); ^{13}C NMR (100MHz, $CDCl_3$) δ 195.3 (C=O), 171.2 (C=O), 162.4 (Ph), 135.4 (Ph), 127.8 (Ph), 114.0 (Ph), 65.8 (PO$\underline{C}$H$_2$CH$_2$CH$_2$CH$_3$), 59.9 ($N_{ring}\underline{C}$H$_2$CON), 56.4 ($N_{ring}\underline{C}$H$_2$CON), 55.1 (b, $N_{ring}\underline{C}$H$_2$), 40.6 (NH$\underline{C}$H$_2$PO), 40.5(NH$\underline{C}$H$_2$PO) 34.2 (d, N$\underline{C}$H$_2$P, J_{CP} = 157 Hz), 32.4 (POCH$_2\underline{C}$H$_2$CH$_2$CH$_3$), 18.6 (POCH$_2$CH$_2\underline{C}$H$_2$CH$_3$), 13.5 (POCH$_2$CH$_2$CH$_2\underline{C}$H$_3$). *m/z* (ESMS ESI+): 1096 $[M+H]^+$, an appropriate isotope pattern was observed.

Synthesis of the Metal Complex

Terbium (III) 1-*N*-[2-[4-hydroxyphenyl]-2-oxoethyl]-4,7,10-tris-(aminomethylphosphonic acid dibutyl ester) amide-1,4,7,10-tetraazacyclododecane (Tb•2). To a solution of the ligand 2 (0.305 g, 0.28 mmol) in acetonitrile (40 mL) was added a solution of the terbium triflate (0.166 g, 0.27 mmol) in acetonitrile (10 mL). The reaction was heated to 70 ^{o}C and stirred for 18 hours. The absence of any free Tb^{3+} metal was confirmed using the Xylenol Orange indicator test. The solvent was removed under

reduced pressure, and the residue was dried under high vacuum for 3 hours to afford the complex. The concentration of Tb^{3+} in this complex was measured using ICP-OES analysis.

VI. References

[1] T. J. Swift, R. E. Connick, *J. Chem. Phys.* **1962**, *37*, 307-320.
[2] a) Y. Wu, T. C. Soesbe, G. E. Kiefer, P. Zhao, A. D. Sherry, *J. Am. Chem. Soc.* **2010**, *132*, 14002-14003; b) F. A. Rojas-Quijano, E. T. Benyo, G. Tircso, F. K. Kalman, Z. Baranyai, S. Aime, A. D. Sherry, Z. Kovacs, *Chemistry* **2009**, *15*, 13188-13200.