Electronic Supporting Information

Terbium(III) Complex as a Luminescent Sensor for Human Serum Albumin in Aqueous Solution

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Contents

1 Experimental

1.1 Reagents and Materials

Common reagents used in the experiments were all of analytical grade. Diethylenetriaminepentaacetic acid (DTPA) dianhydride and 1-(2-aminoethyl)-2-methyl-5-nitroimidazole were purchased from Acros Organics. 2-(2-methyl-1H-imidazol-1-yl)ethanamine was synthesized according to literature.[1] Human serum albumin (96-99% agarose gel electrophoresis, fraction V powder, remainder mostly globulins) was purchased from Sigma. 3-*sn*-phosphatidyl-L-serine (PS) sodium salt from bovine brain, enzymes, proteins, amino acids, and tris(hydroxymethyl)aminomethane (Tris) were also purchased from Sigma. Anhydrous *N*, *N*-dimethylformamide (DMF) was used after distillation and purification.

1.2 Methods

^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer. Electrospray ionization mass spectra (ESI-MS) were acquired on an LCQ Fleet electrospray mass spectrometer. The isotopic distribution patterns for the complexes were simulated using the Isopro 3.0 program. Elemental analysis was performed on a CHN-O-Rapid analyzer (Heraeus, Germany). Time-resolved luminescence spectra were recorded on a Perkin-Elmer LS 55 luminescence spectrometer with the following settings: delay time, 100 μs; gate time, 2.00 ms; and cycle time, 20 ms. The excitation and emission slit widths were 8 nm for TbL and 4 nm for TbL', respectively. The photomultiplier voltages were 900 V for TbL and 700 V for TbL', respectively. UV-vis spectra were determined on a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. Luminescence lifetimes were obtained on a FLS 920 time resolved and steady state fluorescence lifetime spectrometer (Edinburgh, UK).

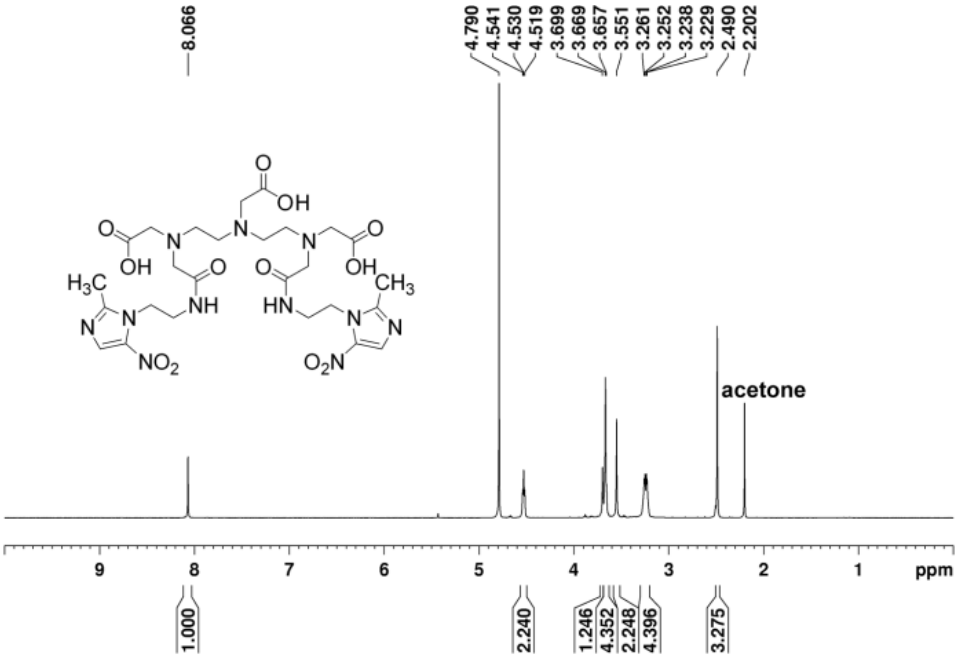
1.3 Synthesis and Characterization of Ligands and Complexes

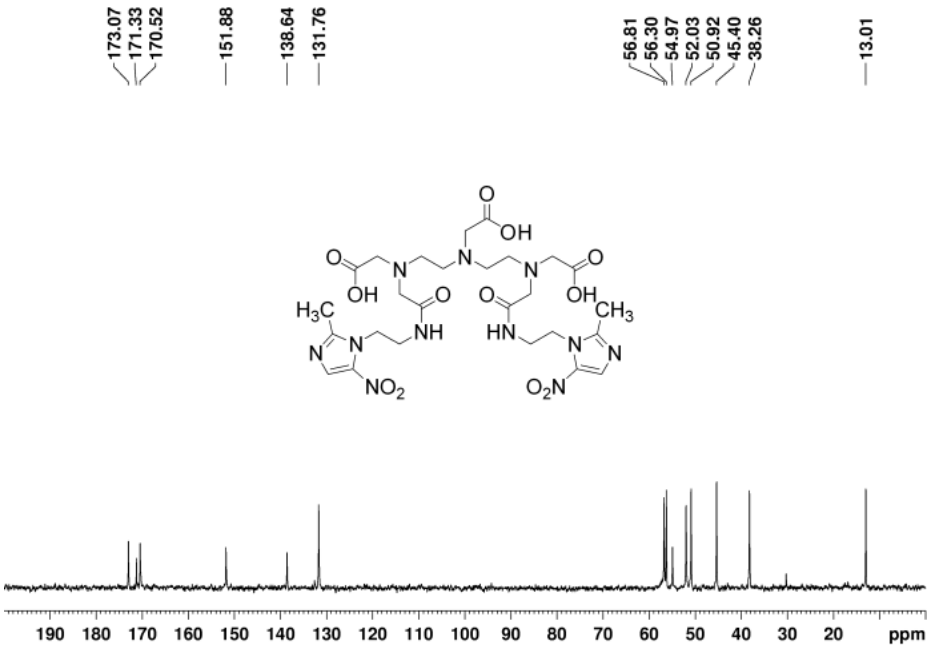
General synthetic route for TbL and TbL':

H_3L: It was synthesized according to the literature.[2] Briefly, 1-(2-aminoethyl)-2-methyl-5-nitroimidazole (3.75 g, 14.4 mmol) in DMF (30 ml) was added dropwise to DTPA dianhydride (2.2 g, 6 mmol) in DMF (150 ml) and triethylamine (6 ml) under stirring in nitrogen at 0 ºC. The ice bath was removed after 2 h and the reaction

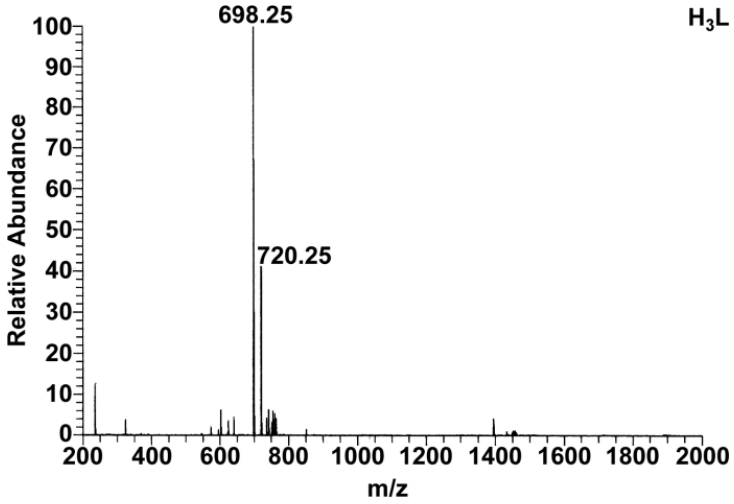
mixture was stirred at room temperature for 48 h. The reaction was quenched by H_2O (150 ml) and the solvent was evaporated to 10 ml. Acetone (200 ml) was added to the residue and the resulting precipitate was filtered, washed with anhydrous chloroform and ether, and dried under vacuum to give H_3L as a white powder (2.8 g, yield: 69%). Elemental analysis found (calcd) for $C_{26}H_{39}N_{11}O_{12}$ (%): C, 45.12 (44.76); H, 5.91 (5.56); N, 21.88 (22.08). ^{1}H NMR (D_2O, 500 MHz, δ, ppm): 2.49 (s, 6H, CH_3-imidazole), 3.25 (m, 8H, NCH_2CH_2N), 3.55 (t, 4H, CH_2-CH_2-imidazole), 3.67 (s, 8H, NCH_2CON), 3.70 (s, 2H, central NCH_2COOH), 4.53 (t, 4H, -CH_2-imidazole), 8.07 (s, 2H, imidazole). ^{13}C NMR (D_2O, 500 MHz, δ, ppm): 13.01, 38.26, 45.40, 50.92, 52.03, 54.97, 56.30, 56.81, 131.76, 138.64, 151.88, 170.52, 171.33, 173.07 ppm. IR (ν_{max}, cm^{-1}): 3408, 1661, 1531, 1468, 1428, 1365, 1262, 1151, 826. ESI-MS found (calcd) for $C_{26}H_{39}N_{11}O_{12}$ (*m/z*): 698.25 (698.29) $[M + H]^+$, 720.25 (720.27) $[M + Na]^+$.

^{1}H and ^{13}C NMR spectra of **H_3L**:



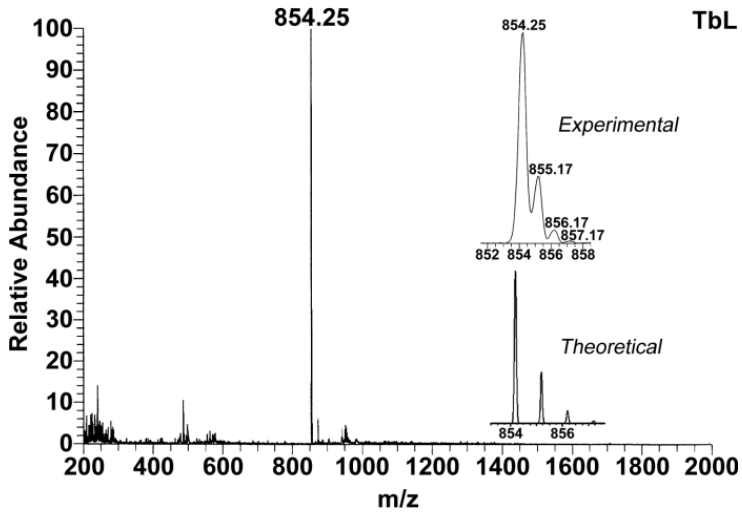


ESI-MS spectrum of **H_3L**:



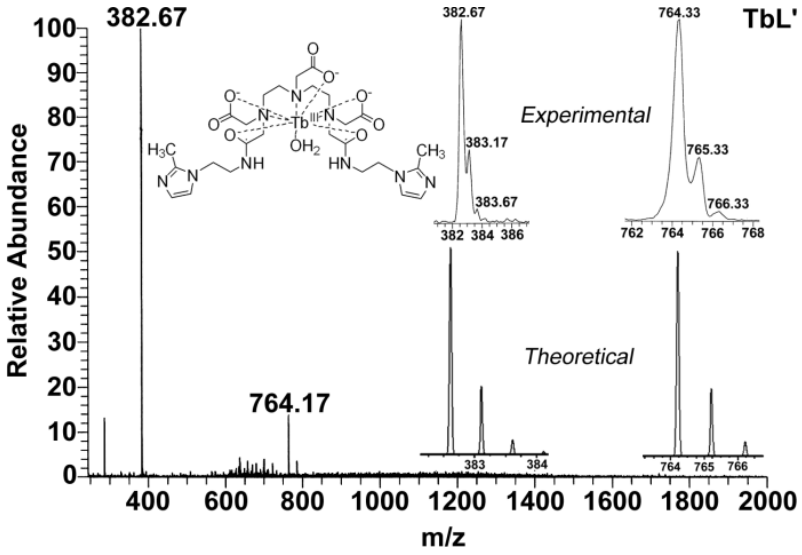
TbL: It was prepared by a modified literature procedure.[3] In brief, NaOH (5 M) was added to H_3L (0.348 g, 0.5 mmol, 15 ml) until the pH reached 6. $Tb(NO_3)_3 \cdot 6H_2O$ (3 ml, 0.226 g, 0.5 mmol) was added to this solution slowly and the pH was maintained at 6.5 by adding aliquots of NaOH (5 M). The mixture was stirred at 45 ºC for 4 h and the solvent was evaporated. The residue was dissolved in distilled water (1 ml) and the solution was kept at room temperature under vigorous stirring for 1 h until the pH was stabilized. The product was purified by precipitation with acetone for 3 times and the obtained light yellow solid was dried in vacuum (0.218 g, yield: 50%). Elemental analysis found (calcd) for $C_{26}H_{38}N_{11}O_{13}Tb$ (%): C, 35.01 (35.83); H, 4.94 (4.39); N, 18.23 (17.68). IR (ν_{max}, cm^{-1}): 3360, 1628, 1468, 1392, 1314, 1262, 1188, 1109, 826. ESI-MS found (calcd) for $C_{26}H_{38}N_{11}O_{13}Tb$ (*m/z*): 854.25 (854.19) $[M - H_2O + H]^+$.

ESI-MS spectrum of **TbL** in H_2O:



TbL': It was prepared by a method as described above for TbL except **H_3L'** was used (yield: 43%). Elemental analysis found (calcd) for $C_{26}H_{40}N_9O_9Tb$ (%): C, 40.56 (39.95); H, 5.03 (5.16); N, 16.64 (16.13). IR (ν_{max}, cm^{-1}): 3416, 1597, 1324, 1094, 933, 720. ESI-MS found (calcd) for $C_{26}H_{40}N_9O_9Tb$ (*m/z*): 382.67 (382.62) $[M - H_2O + 2H]^{2+}$, 764.17 (764.22) $[M - H_2O + H]^{+}$.

ESI-MS spectrum of **TbL'** in H_2O:



2 Supplementary Figures

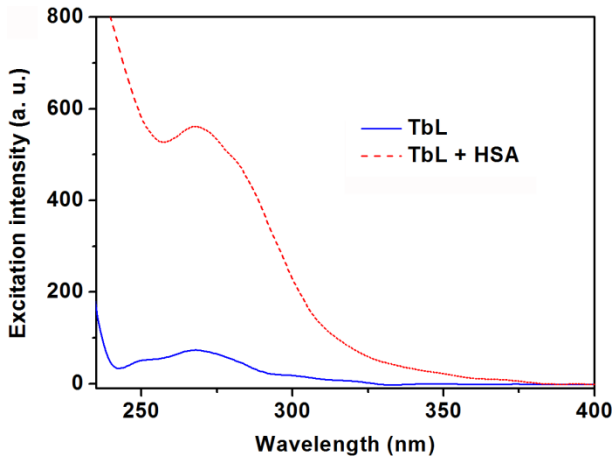


Fig. S1 The excitation spectra of TbL (0.1 mM) in the presence or absence of HSA (20 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 25 ºC.

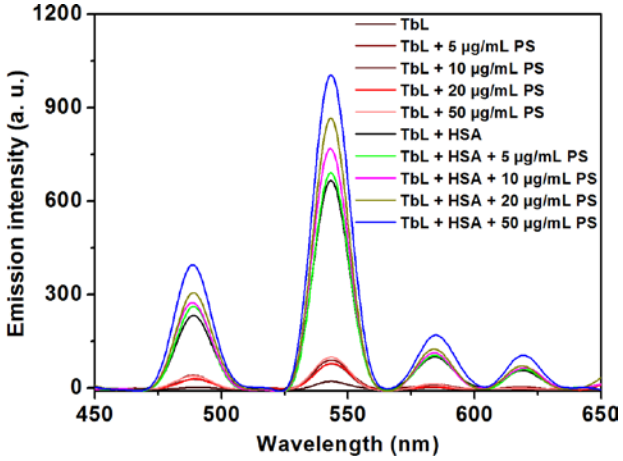


Fig. S2 The time-resolved luminescence spectra of TbL (0.1 mM, λ_{ex} = 265 nm) in the absence or presence of PS, HSA (20 μM) or HSA plus PS and in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) after incubation at 37 °C for 20 min.

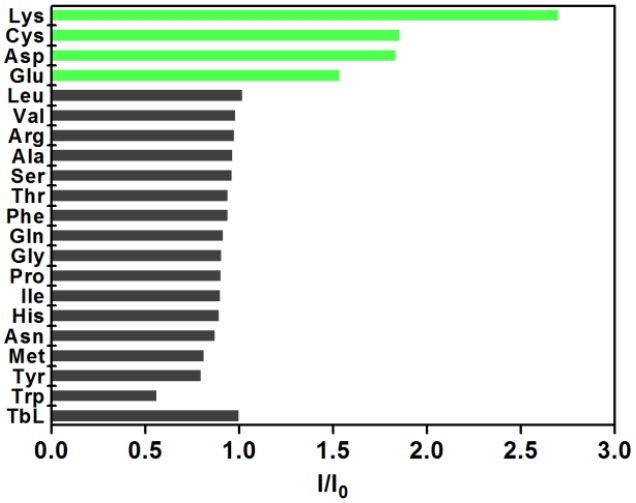


Fig. S3 The time-resolved luminescence emission intensity of TbL (0.1 mM, λ_{ex} = 265 nm) in the presence (I) versus the absence (I_0) of different amino acids (0.4 mM) at 545 nm in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

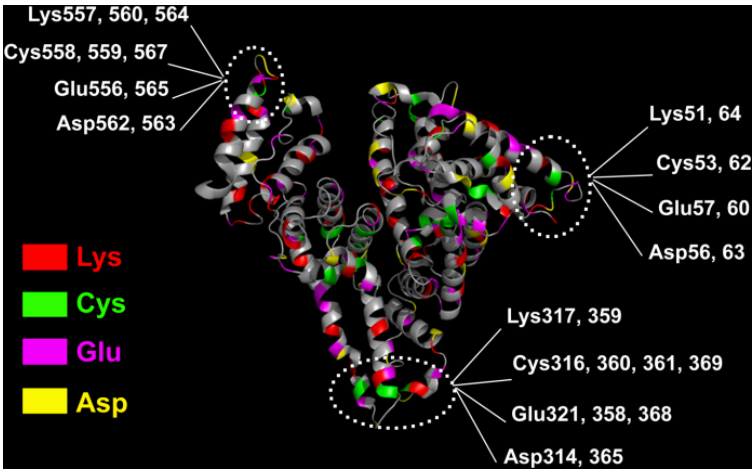


Fig. S4 The distribution of Lys, Cys, Glu, and Asp residues in the structure of HSA (PDB ID: 1UOR,[4] created by software Pymol[5]). The aggregation regions of these residues are highlighted by the circles.

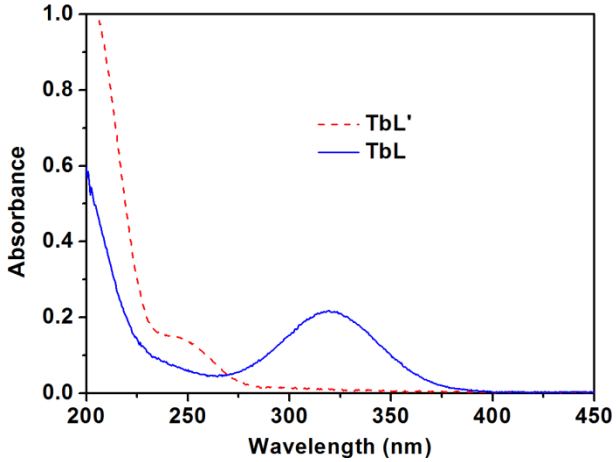


Fig. S5 The absorption spectra of TbL and TbL' (0.1 mM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

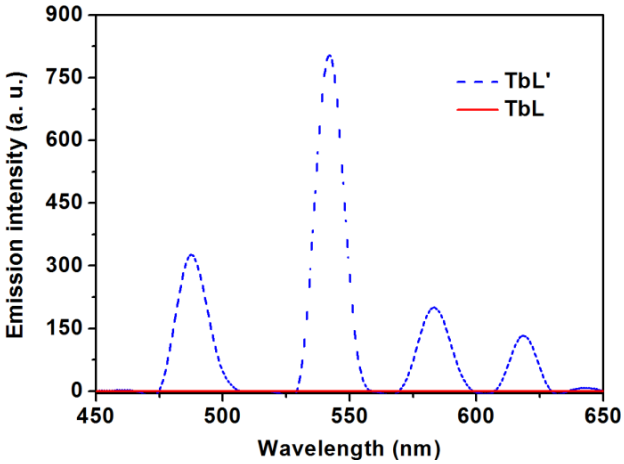


Fig. S6 The time-resolved luminescence emission spectra of TbL and TbL' (0.1 mM, λ_{ex} = 265 nm) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

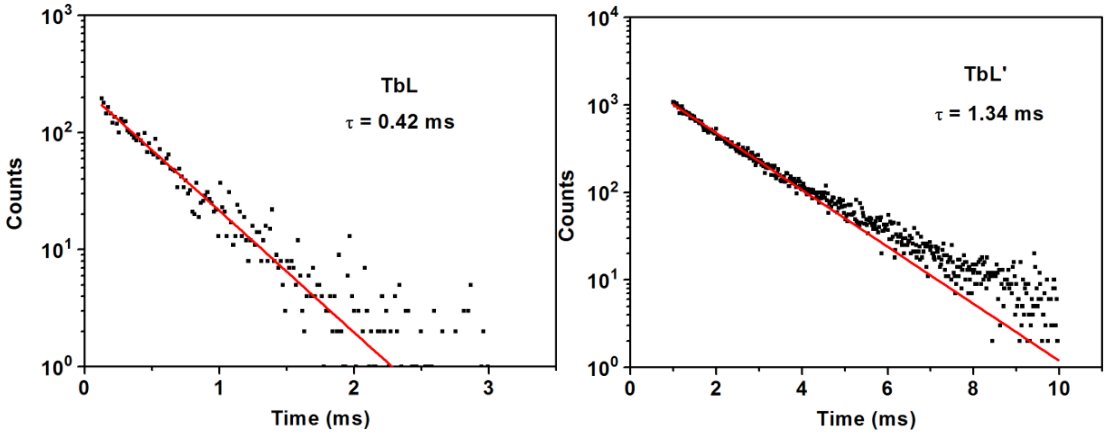


Fig. S7 Decay of the luminescence emission (545 nm) of TbL and TbL' (0.1 mM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) upon excitation at 265 nm and 245 nm, respectively. The data were fitted to a single-exponential curve obeying the equation $I = I_0 \exp(-t/\tau)$,[6] where I_0 and I are luminescence intensities at time 0 and t, respectively, and τ is the luminescence emission lifetime.

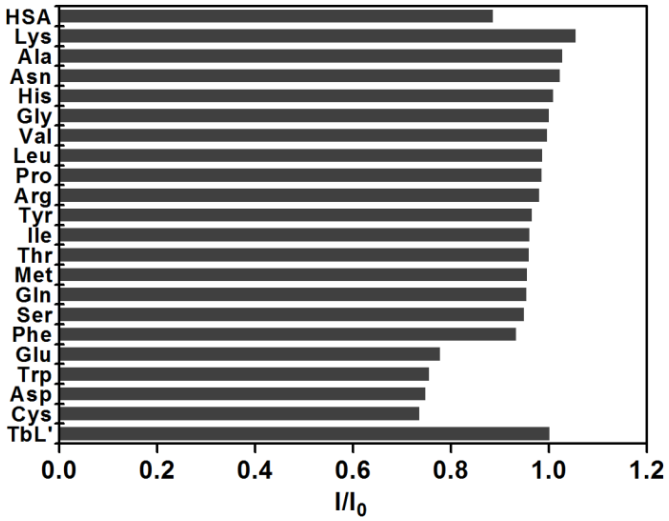


Fig. S8 The ratio of time-resolved luminescence emission intensity of TbL' (0.1 mM) in the presence of HSA (20 μM) or different amino acids (0.4 mM) (I) to that of TbL' alone (I_0) at 545 nm in buffer (λ_{ex} = 245 nm, 5 mM Tris-HCl, 50 mM NaCl, pH 7.4) after incubation at 37 °C for 20 min.

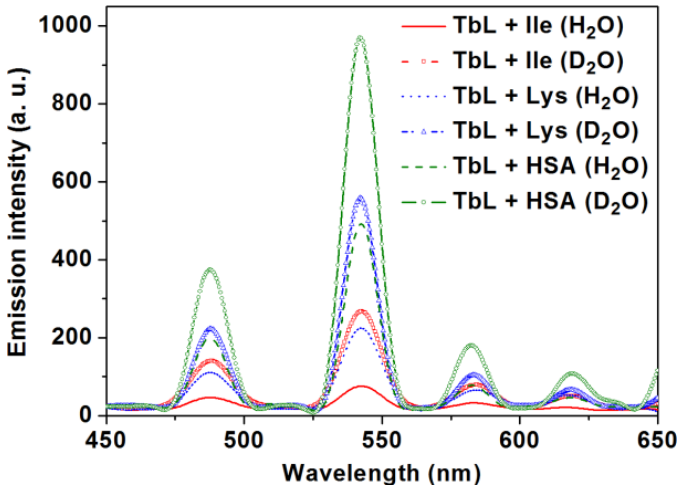


Fig. S9 The time-resolved luminescence emission spectra of TbL (0.1 mM, λ_{ex} = 265 nm) in the presence of Lys, Ile (0.4 mM) or HSA (20 μM) determined in H_2O and D_2O, respectively, after incubation at 37 °C for 20 min.

3 References

[1] M. P. Hay, W. R. Wilson, J. W. Moselen, B. D. Palmer and W. A. Denny, *J. Med. Chem.*, 1994, **37**, 381.
[2] K. Hanaoka, K. Kikuchi, Y. Urano and T. Nagano, *J. Chem. Soc., Perkin Trans.*, 2001, **2**, 1840.
[3] T. Hirayama, M. Taki, A. Kodan, H. Kato and Y. Yamamoto, *Chem. Commun.*, 2009, 3196.
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[6] A. Beeby, I. M. Clarkson, R. S. Dickins, S. Faulkner, D. Parker, L. Royle, A. S. de Sousa, J. A. G. Williams and M. Woods, *J. Chem. Soc., Perkin Trans.* 1999, **2**, 493.