Electronic Supplementary Information

Site-selective Recognition of Peptide Diphosphorylation by a Terbium(III) Complex in Aqueous Solution

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1 Experimental

1.1 Reagents and Materials

Common reagents used in the experiments were all of analytical grade. Diethylenetriaminepentaacetic acid (DTPA) dianhydride and 8-aminoquinoline were purchased from Acros Organics. Guanosine 5′-monophosphate disodium salts (GMP), Guanosine 5′-diphosphate sodium salt (GDP), adenosine 5′-monophosphate disodium salts (AMP), adenosine 5′-diphosphate sodium salt (ATP), adenosine 5′-triphosphate disodium salts (ATP), and tris(hydroxymethyl)aminomethane (Tris) were purchased from Sigma. Tau(231-238) peptides TP1, TP2, TP3, TP4, and TP5 were purchased from GL Biochem Ltd. C57BL/6J (B6) mice were purchased from Model Animal Research Center of Nanjing University. Anhydrous *N*, *N*-dimethylformamide (DMF) was used after distillation and purification.

1.2 Methods

^{1}H, ^{13}C, and ^{31}P 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). UV-vis spectra were determined on a Shimadzu UV-3600 UV-VIS-NIR spectrophotometer. Time-resolved luminescence spectra were recorded on a HORIBA Fluoromax-4P luminescence spectrometer with the following settings: delay time, 50 μs; gate time, 2.00 ms; excitation slit, 5 nm; and emission slit 5 nm.

1.3 Synthesis and Characterization of Ligand and Complex

H_3L: It was synthesized according to the literature.[1] Briefly, 8-aminoquinoline (2.08 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 ℃. 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 pale powder (2.9 g, yield: 76%). Elemental analysis found (calcd) for $C_{32}H_{35}N_7O_8$ (%): C, 59.12 (59.53); H, 5.23 (5.46); N, 15.94 (15.19). ^{1}H NMR (D_2O, 500 MHz, δ, ppm): 3.375 (br, 4H, NCH_2CH_2N), 3.609 (br, 4H, NCH_2CH_2N), 3.691 (s, 4H, terminal NCH_2COOH), 3.863 (s, 4H, NCH_2CON), 4.062 (s, 2H, central NCH_2COOH), 7.344−7.475 (t, 2H, quinoline), 7.558−7.574 (d, 2H, quinoline), 7.621−7.646 (dd, 2H, quinoline), 7.714−7.729 (d, 2H, quinoline), 8.419−8.436 (d, 2H, quinoline), 8.768−8.777 (d, 2H, quinoline). ^{13}C NMR (D_2O, 125 MHz, δ, ppm): 49.96, 53.00, 55.57, 58.88, 120.00, 121.90, 124.34, 126.67, 127.85, 130.88, 137.57, 137.99, 148.59, 170.61, 171.52, 177.75 ppm. ESI-MS found (calcd) for $C_{32}H_{35}N_7O_8$ (*m/z*): 644.50

(644.67) $[M - H]^-$, 682.33 (682.76) $[M + K - 2H]^-$.

TC: It was prepared by a modified literature procedure.[2] In brief, NaOH (5 M) was added to H_3L (0.323 g, 0.5 mmol, 15 ml) until the pH reached 6. $Tb(NO_3)_3$ $6H_2O$ (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 ℃ 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 (0.184 g, yield: 45%). Elemental analysis found (calcd) for $C_{32}H_{34}N_7O_9Tb$ (%): C, 46.14 (46.90); H, 4.03 (4.18); N, 12.17 (11.96). IR (ν_{max}, cm^{-1}): 3408, 2988, 1687, 1601, 1400, 1231, 1099, 964. ESI-MS found (calcd) for $C_{32}H_{34}N_7O_9Tb$ (*m/z*): 824.42 (824.56) $[M - H_2O + Na]^+$.

1.4 Luminescence and absorption titrations of TC with Zn(II) ion.

TC (25 μM) and $ZnCl_2$ (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, and 75 μM, respectively) were mixed in Tris-HCl buffer and incubated at 37 ℃ for 30 min. The time-resolved luminescence and absorption spectra of the resulting solutions were measured at 25 ℃. The luminescence or absorbance intensity of TC was plotted against the concentration of Zn(II) at 545 and 250 nm, respectively. The stoichiometry of the reaction was calculated by nonlinear least-squares fitting to the titration curve of TC with Zn(II) with equation 1.[3]

$$F = (F_0 + F_{max} \times K \times ([Zn])^n)/(1 + K \times ([Zn])^n) \quad 1$$

Where F is the luminescence or absorbance intensity, F_0 is the intensity without Zn(II), F_{max} is the maximum intensity in the presence of Zn(II), n the is the binding stoichiometry, and K is the association constant. The values of n were determined to be 2.1 and 1.9 from the fittings for the data shown in Fig. S4 and S5, respectively.

1.5 Reactions of TC with peptides or anions in the presence of Zn(II).

TC (25 μM) was preincubated with $ZnCl_2$ (50 μM) in Tris-HCl buffer at 37 ℃ for 30 min. After that, different amount of peptides or anions were added to the above solution and coincubated at 37 ℃ for 10 min, respectively. The time-resolved luminescence or absorption spectra of the resulting solutions were measured at 25 ℃.

1.6 Minimum detection limit and linear range of TC for TP3.

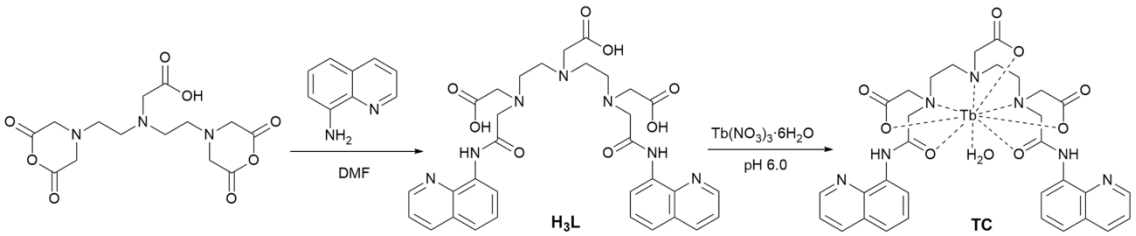
The time-resolved luminescence spectra of TC (25 μM, λ_{ex} = 250 nm) in the presence of Zn(II) (50 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) was collected for 20 times to determine the background noise σ.[4] The time-resolved luminescence spectra of TC upon addition of CT-DNA at various concentration in the presence of Zn(II) was measured. A linear regression curve was fitted according to the emission intensity at 545 nm in the range of 0 − 1 μM, and the slope of curve was obtained using *Origin* 8.5.

1.7 Time-resolved luminescence measurement in brain homogenates of mice.

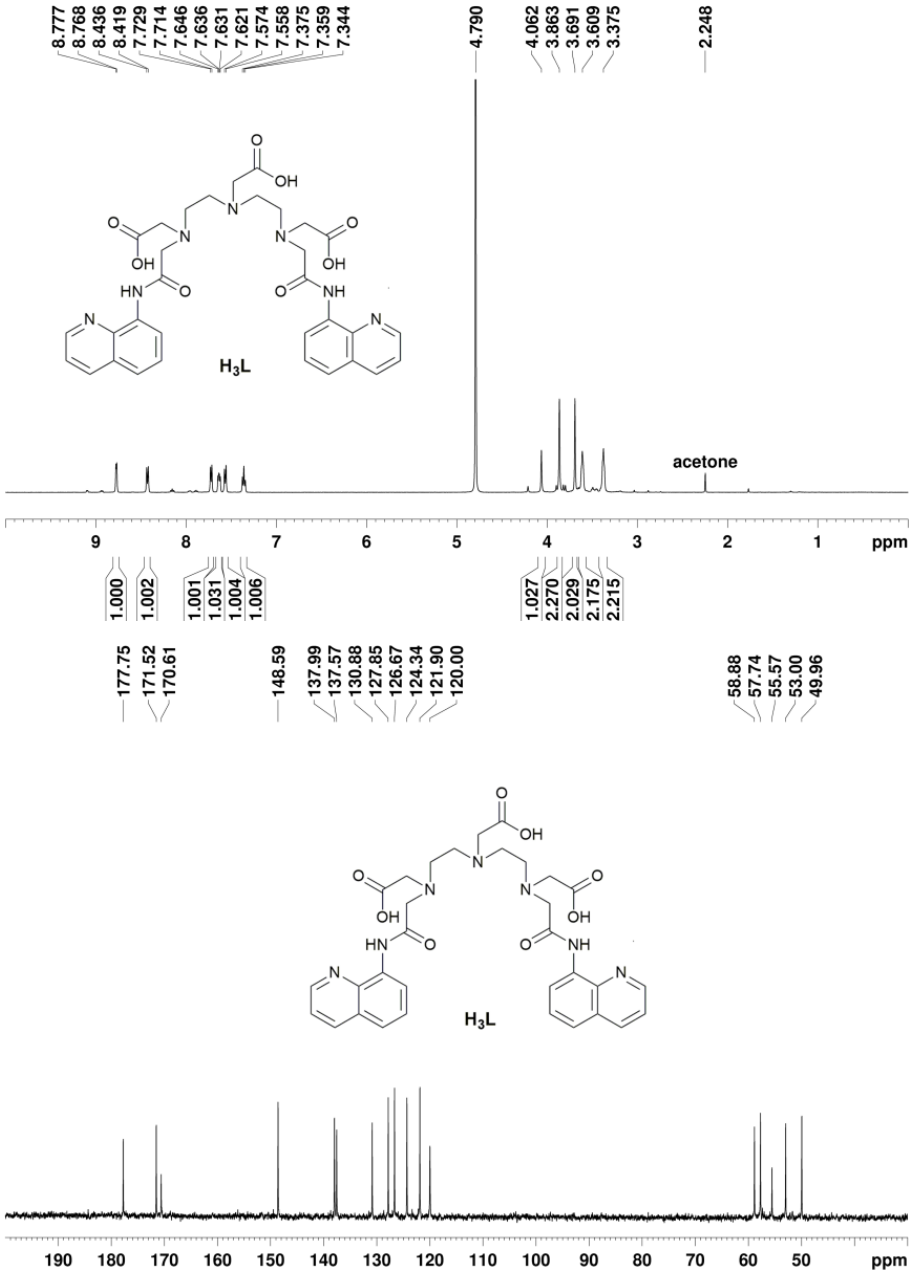
Brain extracts of C57BL/6J mice were prepared according to the reported procedure.[5] The extracted tissue pieces were homogenized in lysis buffer (Tris-HCl, 50 mM; NaCl, 150 mM; NP-40, 1%; Na-deoxycholate, 0.25%; PMSF, Na_3VO_4, NaF, 1 mM each; aprotinin, leupeptin, pepstatin, 1 μg/mL each). The homogenate solution was centrifuged at 12000 rpm for 10 min at 4 ℃ and the resulting supernatant was collected. The time-resolved luminescence spectra of TPC

(25 μM) upon reacting with TP3 (5 μM) in the presence of Zn(II) (50 μM) were measured in the same way as in Tris-HCl buffer described above. The delay time was set to 0.025, 0.05, 0.1, 0.15, and 0.2 ms, respectively.

2 Supplementary Figures



Scheme S1 Synthesis of TC.



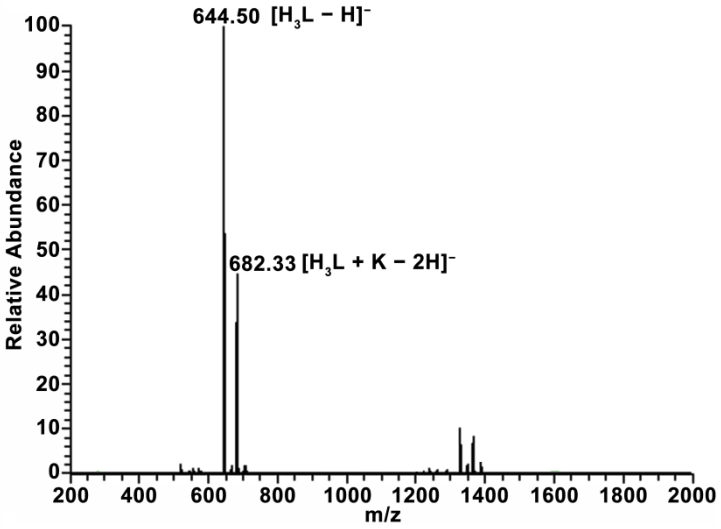


Fig. S1 ^{1}H NMR (D_2O), ^{13}C NMR (D_2O), and ESI-MS spectra for H_3L.

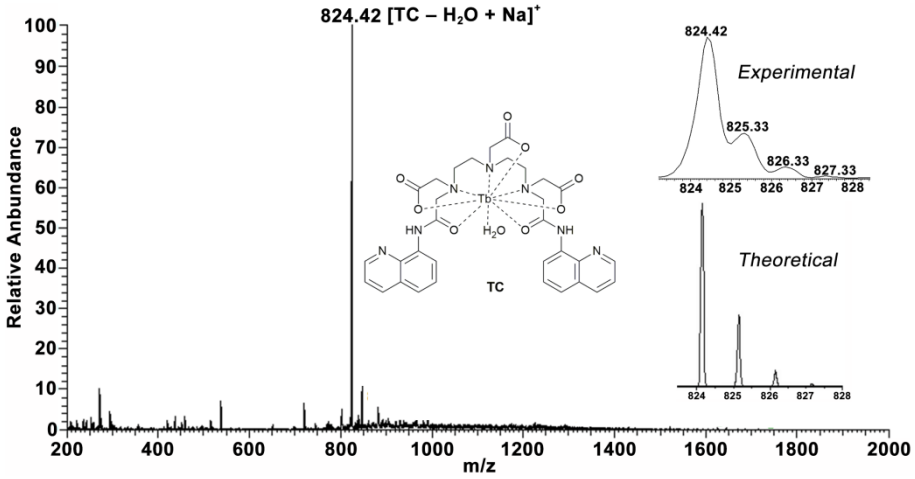


Fig. S2 ESI-MS spectra for TC.

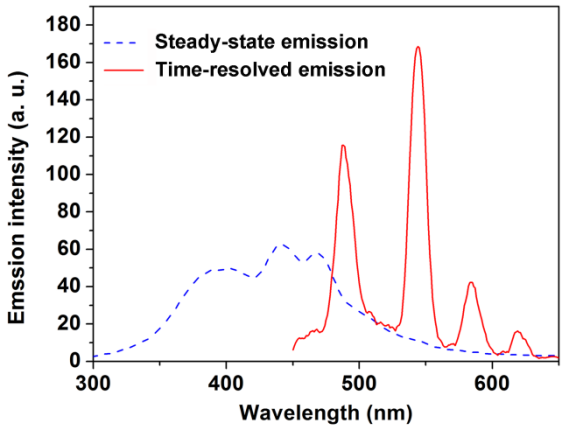


Fig. S3 Steady-state and time-resolved (delay time = 50 μs) emission spectra of TC (25 μM, λ_{ex} = 250 nm) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

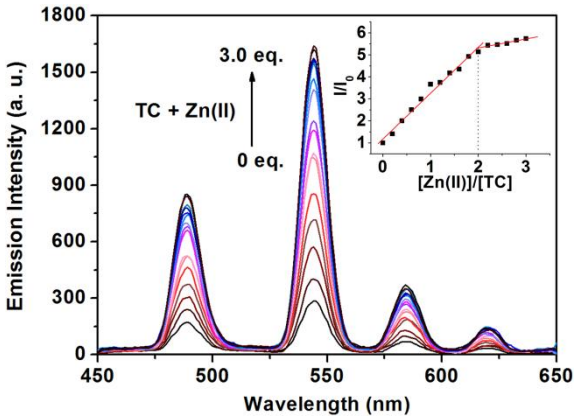


Fig. S4 The time-resolved luminescence spectra of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) upon addition of increasing concentration of Zn(II) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4). Inset shows the emission intensity ratio (I/I_0) at 545 nm of TC versus different [Zn(II)]/[TC] ratio.

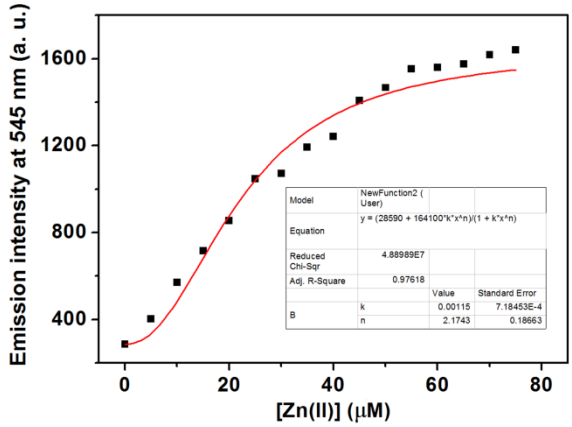


Fig. S5 A nonlinear least-squares curve fitness with respect to the intensity of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) at 545 nm as a function of Zn(II) concentration. The value of binding stoichiometry (*n*) was determined to be 2.1.

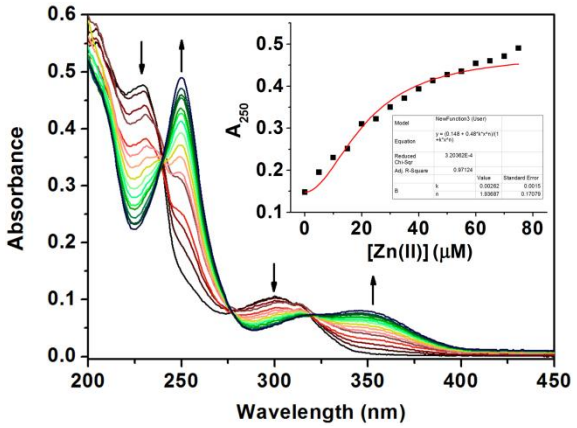


Fig. S6 UV-vis absorption spectra of TC (25 μM) upon addition of increasing concentration of Zn(II) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4). Inset shows the nonlinear least-squares curve-fit of absorption intensity at 250 nm as a function of Zn(II) concentration. The value of binding stoichiometry (*n*) was determined to be 1.9.

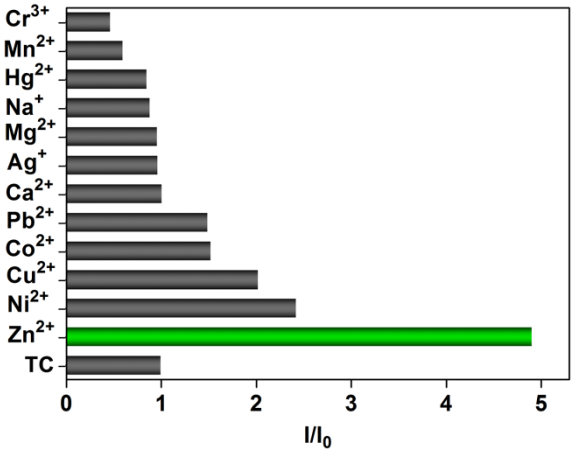


Fig. S7 The luminescence intensities of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) at 545 nm in the presence (I) *vs*. the absence (I_0) of different metal ions (50 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

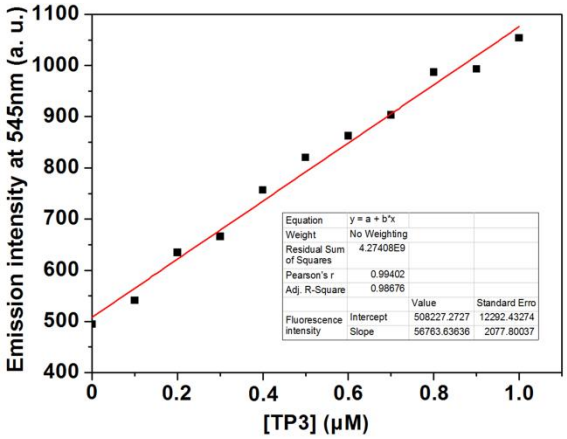


Fig. S8 Plot of luminescence intensity of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) in the presence of Zn(II) (50 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 545 nm as a function of TP3 concentration in the range of 0 − 1 μM.

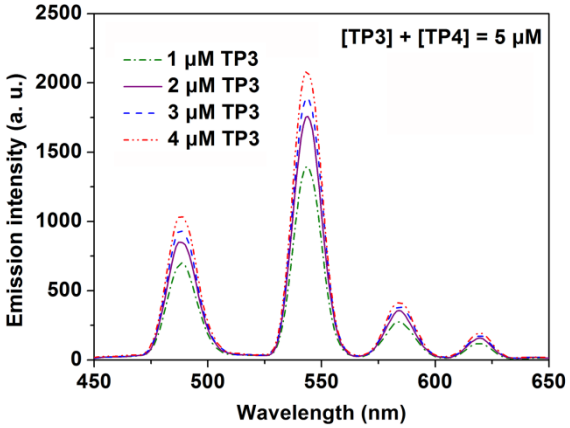


Fig. S9 The time-resolved luminescence responses of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) to varying [TP3]/[TP4] ratios in the presence of Zn(II) (50 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

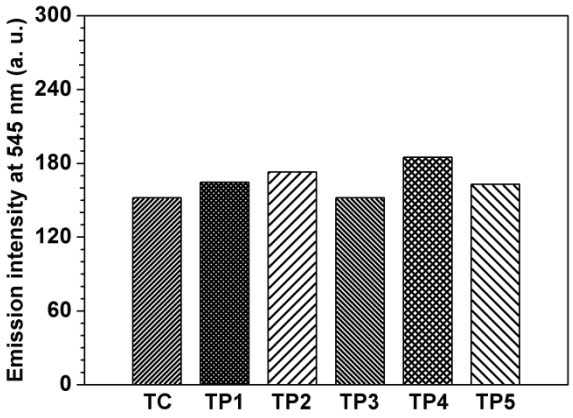


Fig. S10 The luminescence intensities of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) at 545 nm in response to different peptides (5 μM) in the absence of Zn(II) (50 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

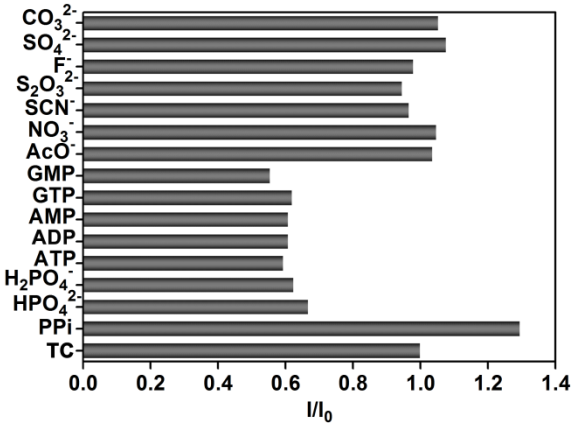


Fig. S11 The luminescence intensities of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) at 545 nm in the presence (I) *vs*. the absence (I_0) of different anions (50 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

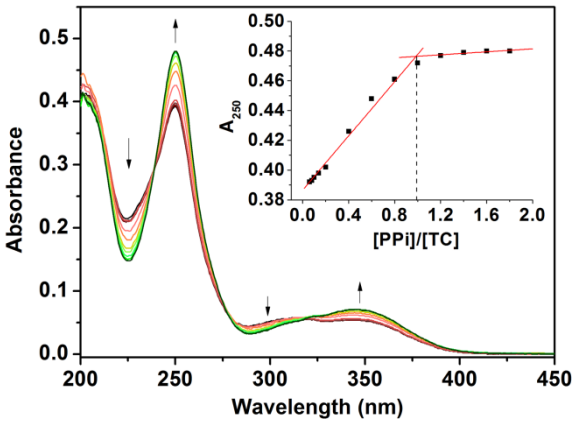


Fig. S12 UV-vis absorption spectra of TC (25 μM) upon addition of increasing concentration of PPi in the presence of Zn(II) (50 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4). Inset shows the absorption intensities of TC-Zn(II) at 250 nm versus different [PPi]/[TC] ratio.

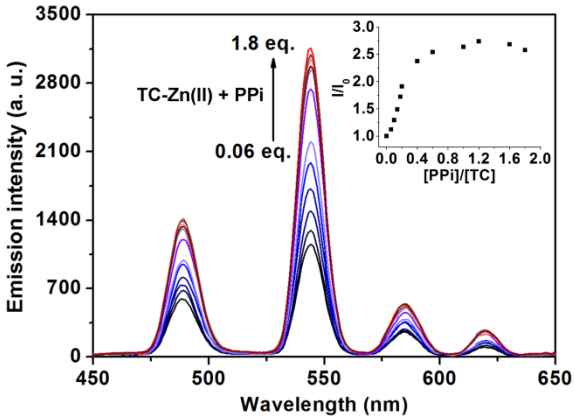


Fig. S13 The time-resolved luminescence spectra of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) upon addition of increasing concentration of PPi in the presence of Zn(II) (50 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) after incubation at 37 ℃ for 10 min. Inset shows the emission intensity ratio (I/I_0) at 545 nm of TC versus different [PPi]/[TC] ratio.

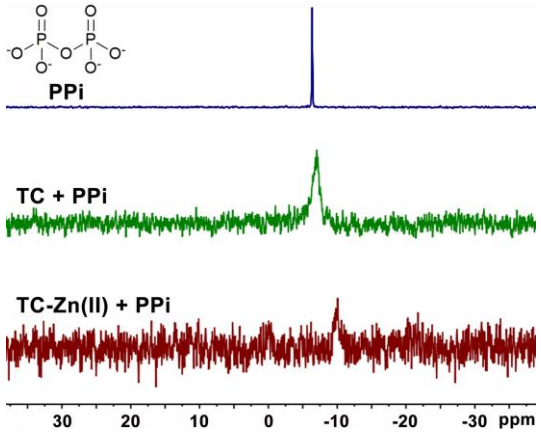


Fig. S14 ^{31}P NMR spectra of PPi (0.5 mM) in the absence and presence of equivalent amounts of TC or TC-Zn(II) in D_2O.

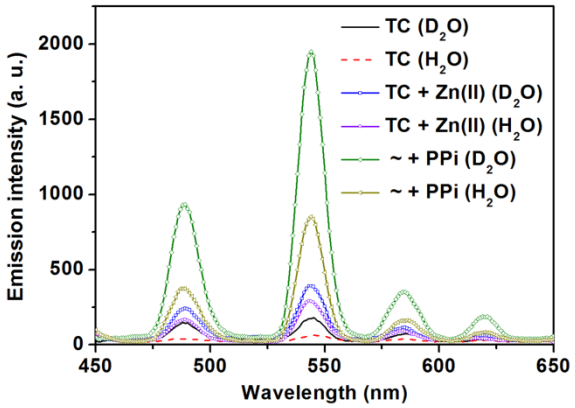


Fig. S15 The time-resolved luminescence spectra of TC (25 μM, λ_{ex} = 250 nm, delay time = 50 μs) in the presence and absence of Zn(II) (50 μM) or PPi (25 μM) determined in H_2O and D_2O.

3 References

(1) K. Hanaoka, K. Kikuchi, Y. Urano and T. Nagano, *J. Chem. Soc., Perkin Trans.*, 2001, **2**, 1840.

(2) X. H. Wang, X. Y. Wang, Y. Q. Wang and Z. J. Guo, *Chem. Commun.*, 2011, **47**, 8127
(3) C. R. Lohani, J. -M. Kim, S. -Y. Chung, J. Y. Yoon and K. -H. Lee, *Analyst*, 2010, **135**, 2079.
(4) X. H. Wang, X. Y. Wang, S. S. Cui, Y. Wang, G. J. Chen and Z. J. Guo, *Chem. Sci.*, 2013, **4**, 3748.
(5) T. Bolmont, F. Clavaguera, M. Meyer-Luehmann, M. C. Herzig, R. Radde, M. Staufenbiel, J. Lewis, M. Hutton, M. Tolnay and M. Jucker, *Am. J. Pathol*., 2007, **171**, 2012.