Electronic Supplementary Information

Specific Recognition of DNA Depurination by a Luminescent Terbium(III) Complex

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1 Experimental

1.1 Materials and Methods

Common reagents used in the experiments were all of analytical grade. TbL and TbL′ were prepared as reported previously.[1] MMS was purchased from Alfa Aesar. Tris(hydroxymethyl)aminomethane (Tris), EB, CT-DNA, GMP, AMP, CMP, and TMP were purchased from Sigma. Oligonucleotides ploy(G), ploy(A), ploy(C), ploy(T), ON1, ON2, and ON3 were purchased from GenScript Co. UV-vis spectra were determined on a Perkin-Elmer Lambda 35 UV-vis spectrometer. 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 12 nm for TbL and 5 nm for TbL′, respectively. The photomultiplier voltage was 900 V. CD spectra were recorded on a Jasco J-810 spectropolarimeter. The concentrations of oligonucleotides were measured by Thermo Scientific NanoDrop 1000. The concentration of CT-DNA was determined by measuring the UV absorption at 260 nm using the known molar absorption coefficient of 6600 M^{-1} L cm^{-1}. The ratio of the UV absorption at 260 and 280 nm in Tris-HCl buffer is around 1.83, indicating that DNA was sufficiently free of protein.[2]

1.2 Reactions of TbL with nucleotides, oligonucleotides or CT-DNA

All the reactions were carried out in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 37 °C for 10 min. The time-resolved luminescence spectra of TbL (100 μM) upon reacting with GMP, AMP, CMP, TMP, ploy(G), ploy(A), ploy(C), ploy(T), ON1, ON2, ON3, and CT-DNA, respectively, were measured at 25 °C after excitation at 260 nm. The time-resolved luminescence spectra of TbL′ upon reacting with CT-DNA were measured similarly.

1.3 Reactions of TbL with CT-DNA in the presence of MMS

CT-DNA (90 μM) was incubated with MMS (0.9, 1.35, 1.8, 3.6, 7.2 mM, respectively) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 37 °C for 24 h. TbL (100 μM) was added to the solution and incubated at 37 °C for 10 min. The time-resolved luminescence spectra were measured at 25 °C after excitation at 260 nm.

1.4 Reactions of TbL with CT-DNA in acidic conditions

The depurination of DNA induced by acid was prepared according to the literature.[3] CT-DNA (0.9 mM) in Tris-HCl buffer (pH 7.4) was adjusted with HCl (0.1 M) to obtain a final pH of 5 or 4.5 at 25 °C. Depurination of DNA was achieved by heating the samples for different times at 70 °C. Depurianted DNA was added to the buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) of TbL (100 μM) at 37 °C for 10 min (DNA final concentration: 45 μM). The time-resolved

luminescence spectra were measured at 25 °C after excitation at 265 nm.

1.5 Response of TbL to CT-DNA in the presence of denaturants

CT-DNA was incubated with common DNA denaturants formamide and urea (2 mM) at 90 °C for 5 min.[4] The solution was then cooled with ice and added to the buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) of TbL (100 μM) at 37 °C and reacted for 10 min ($[DNA]_{final}$ = 90 μM). The time-resolved luminescence spectra were measured at 25 °C after excitation at 265 nm.

1.6 Minimum detection limit and linear range of TbL for CT-DNA

The time-resolved luminescence spectra of free TbL (0.1 mM, λ_{ex} = 265 nm) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) was collected for 20 times to determine the background noise σ.[5] The time-resolved luminescence spectra of TbL in the presence of CT-DNA at various concentration was measured. A linear regression curve was fitted according to the emission intensity at 545 nm in the range of 0.08 − 10 μM, and the slope of curve was obtained using *Origin* 8.0.

1.7 DNA binding mode of TbL

CT-DNA (100 μM) was incubated with TbL (10, 20, 40, 60, 80, and 100 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 37 °C for 12 h. CD spectra were measured at 25 °C in the wavelength range of 220−320 nm.

The fluorescence of the EB-DNA system was determined in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 25 °C. CT-DNA (12 μL, 0.9 mM) was added to EB buffer (1.3 μM, 3 mL). Aliquots of TbL (3 μL, 1 mM) were then added to the EB-DNA system at 25 °C in 1 min and the fluorscence spectra were measured on the Perkin-Elmer LS 55 (λ_{ex} = 495 nm, λ_{em} = 610 nm). The apparent binding constant (K_{app}) was determinated by the following equation

$$K_{EB}[EB] = K_{app}[complex]$$

where $K_{EB} = 1.0 \times 10^7$ M^{-1}, [EB] = 1.3 μM, and [complex] was the final concentration at which a 50% reduction of the fluorescence had occurred.[6]

1.8 MD simulation

The initial coordinate of the DNA sequence was generated by using the HyperChem program.[7] The structure of TbL was optimized by Gaussian09 program at the density functional theory (DFT) level.[8] All molecular dynamics (MD) simulations were carried out using the AMBER9 package with the AMBER force fields of ff03 and gaff.[9] The equilibration time for each simulation was 200 ps (NPT) followed by 20 ns of data collection for analysis under the conditions of constant volume with a periodic boundary at 300 K. The ptraj program from AMBER9 simulation software was used to analyze the trajectory.

2 Supplementary Figures

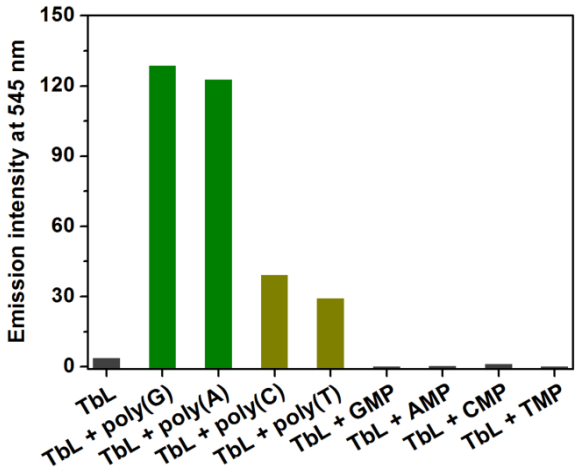

Fig. S1 The time-resolved luminescence intensity of TbL (0.1 mM, λ_{ex} = 265 nm) at 545 nm in the absence or presence of oligonucleotides or nucleotides (40 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

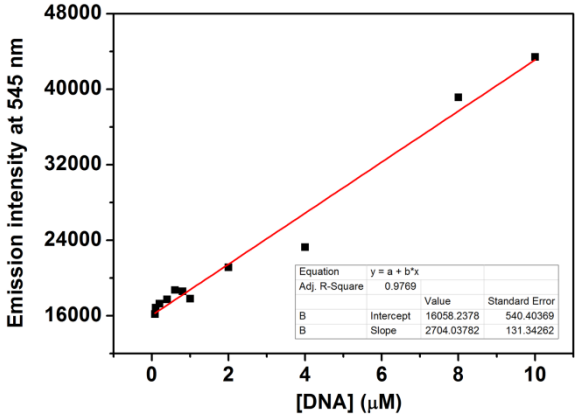

Fig. S2 Plot of luminescence intensity of TbL (0.1 mM, λ_{ex} = 265 nm) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) at 545 nm as a function of CT-DNA concentration in the range of 0.08 − 10 μM.

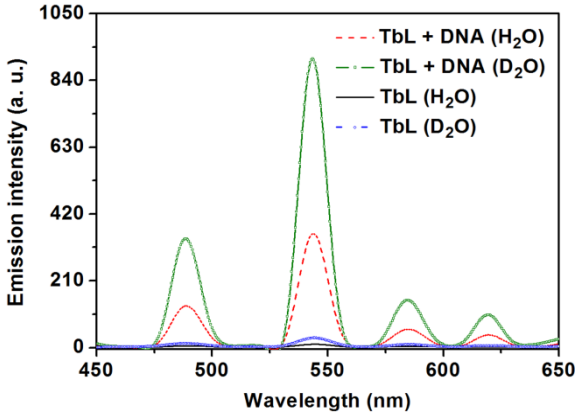

Fig. S3 The time-resolved luminescence spectra of TbL (0.1 mM, λ_{ex} = 265 nm) in the presence and absence of CT-DNA (45 μM) determined in H_2O and D_2O.

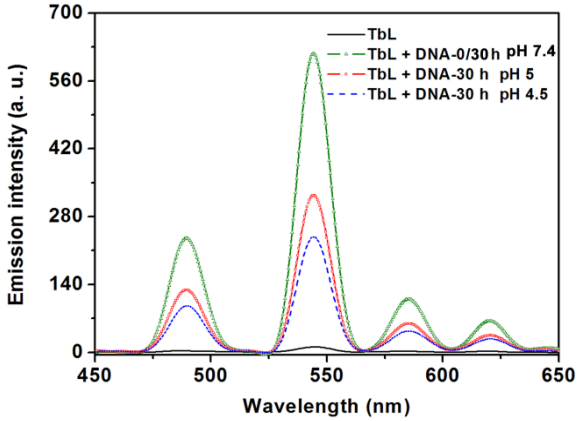

Fig. S4 The time-resolved luminescence spectra of TbL (0.1 mM, λ_{ex} = 265 nm) in the absence or presence of DNA after incubation at 70 °C for 0 or 30 h at different pH values.

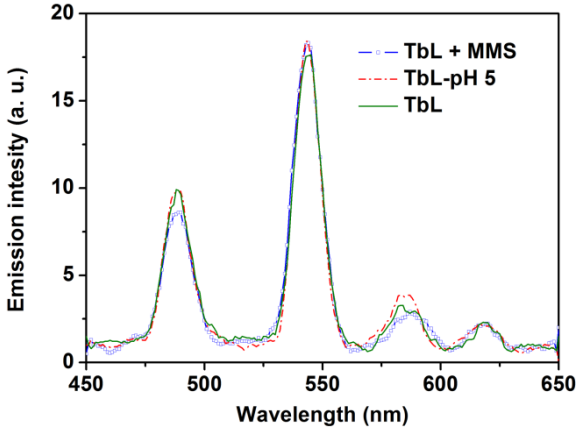

Fig. S5 The effect of MMS (0.9 mM, pH 7.4) or acid (pH 5.0) on the time-resolved luminescence spectra of TbL (0.1 mM, λ_{ex} = 265 nm) in the absence of DNA in buffer (5 mM Tris-HCl, 50 mM NaCl).

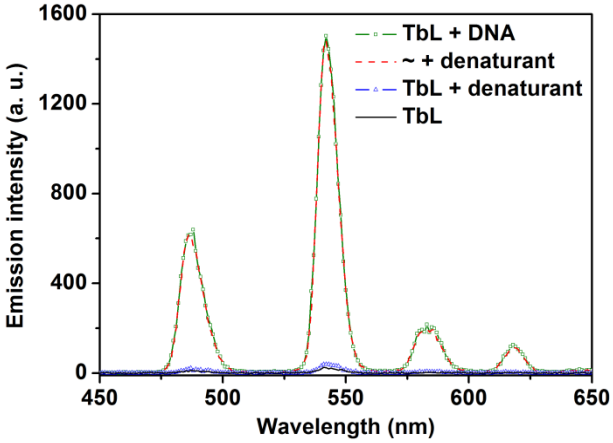

Fig. S6 The time-resolved luminescence spectra of TbL (0.1 mM, λ_{ex} = 265 nm) in the absence or presence of CT-DNA (90 μM) with or without denaturants formamide (2 mM) and urea (2 mM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

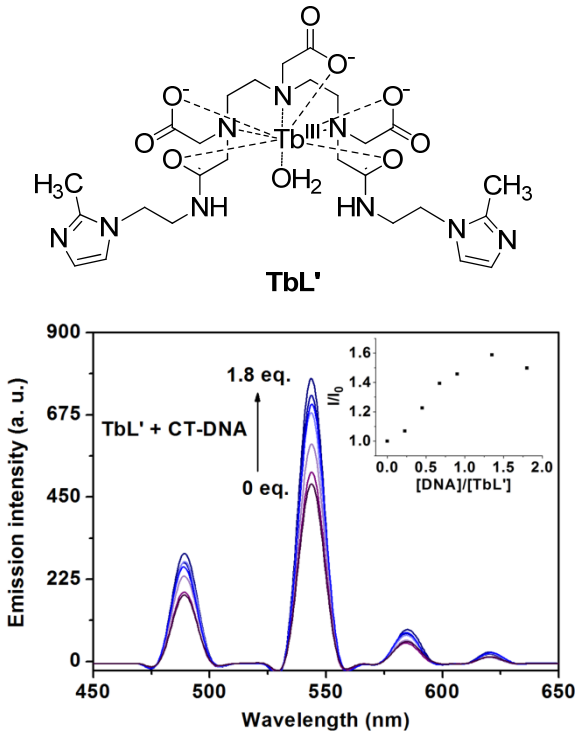

Fig. S7 The structure of TbL′ and its time-resolved luminescence spectra (0.1 mM, λ_{ex} = 265 nm) upon addition of increasing amount of CT-DNA in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4) after incubation at 37 °C for 10 min. Inset shows the emission intensity ratio (I/I_0) at 545 nm versus the ratio of [DNA] to [TbL′].

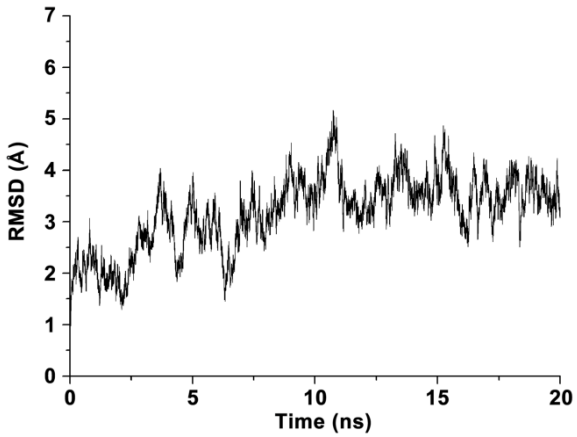

Fig. S8 RMSD values of all backbone atoms in the simulation of TbL-DNA model with respect to the starting structure.

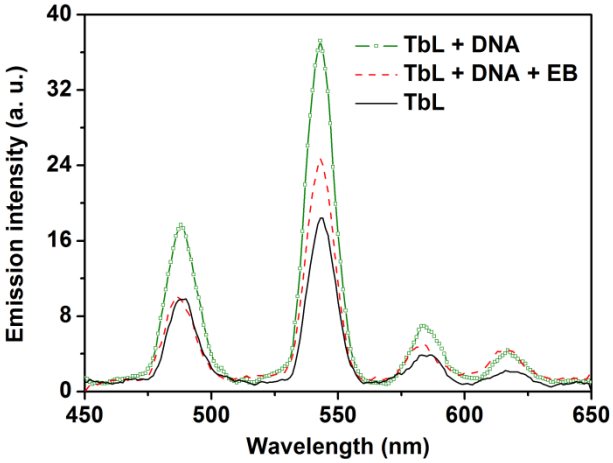

Fig. S9 The time-resolved luminescence spectra of TbL (0.1 mM, λ_{ex} = 265 nm) in response to CT-DNA (3.6 μM) in the absence or presence of EB (1.3 μM) in buffer (5 mM Tris-HCl, 50 mM NaCl, pH 7.4).

Table S1 Parameters for the π-π interactions between metronidazole plane of TbL and purine bases of DNA derived from the MD simulations.

π-π interaction	Distance	Dihedral angle
	Single-binding mode	
Metronidazole − G6	3.878 Å	9.645°
	Double-binding mode	
Metronidazole − G6	3.556 Å	11.988°
Metronidazole − A16	3.773 Å	32.258°

Table S2 Occupancies of hydrogen bonds between oxygen atoms in nitro groups of TbL and N-H groups of DNA bases.

H-bonds	Distance	Occupancy
	Single-binding mode	
A16/N6 − H61 ··· O86	3.0408 Å	83.33%
A16/N6 − H61 ··· O87	3.9651 Å	8.54%
	Double-binding mode	
T5/N3 − H3 ··· O86	2.9336 Å	47.82%
T5/N3 − H3 ··· O87	4.0885 Å	39.26%

References

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