Electronic Supporting Information

A luminescence switch-on probe for terminal deoxynucleotidyl transferase (TdT) activity detection by using an iridium(III)-based i-motif probe

Lihua Lu,[a] Modi Wang,[a] Li-Juan Liu,[b] Chun-Yuen Wong,[c] Chung-Hang Leung*[b] and Dik-Lung Ma*[a,d]

[a] *Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China. E-mail: edmondma@hkbu.edu.hk*

[b] *State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China. E-mail: duncanleung@umac.mo*

[c] *Department of Biology and Chemistry, City University of Hong Kong.*

[d] *Partner State Key Laboratory of Environmental and Biological Analysis, Hong Kong Baptist University, Hong Kong, China.*

Experimental section

Materials. TdT, dCTP, dTTP, dATP and dNTP were purchased from New England Biolabs Inc. (Beverly, MA, USA) and were stored at –20 °C before use. Iridium chloride hydrate ($IrCl_3.xH_2O$) was purchased from Precious Metals Online (Australia). Other reagents, unless specified, were purchased from Sigma Aldrich (St. Louis, MO). All oligonucleotides were synthesized by Techdragon Inc. (Hong Kong, China)

General experimental. Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Deuterated solvents for NMR purposes were obtained from Armar and used as received. Circular dichroism (CD) spectra were collected on a JASCO-815 spectrometer.

^1H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^1H) and 100 MHz (^{13}C). ^1H and ^{13}C chemical shifts were referenced internally to solvent shift (acetone-d_6:

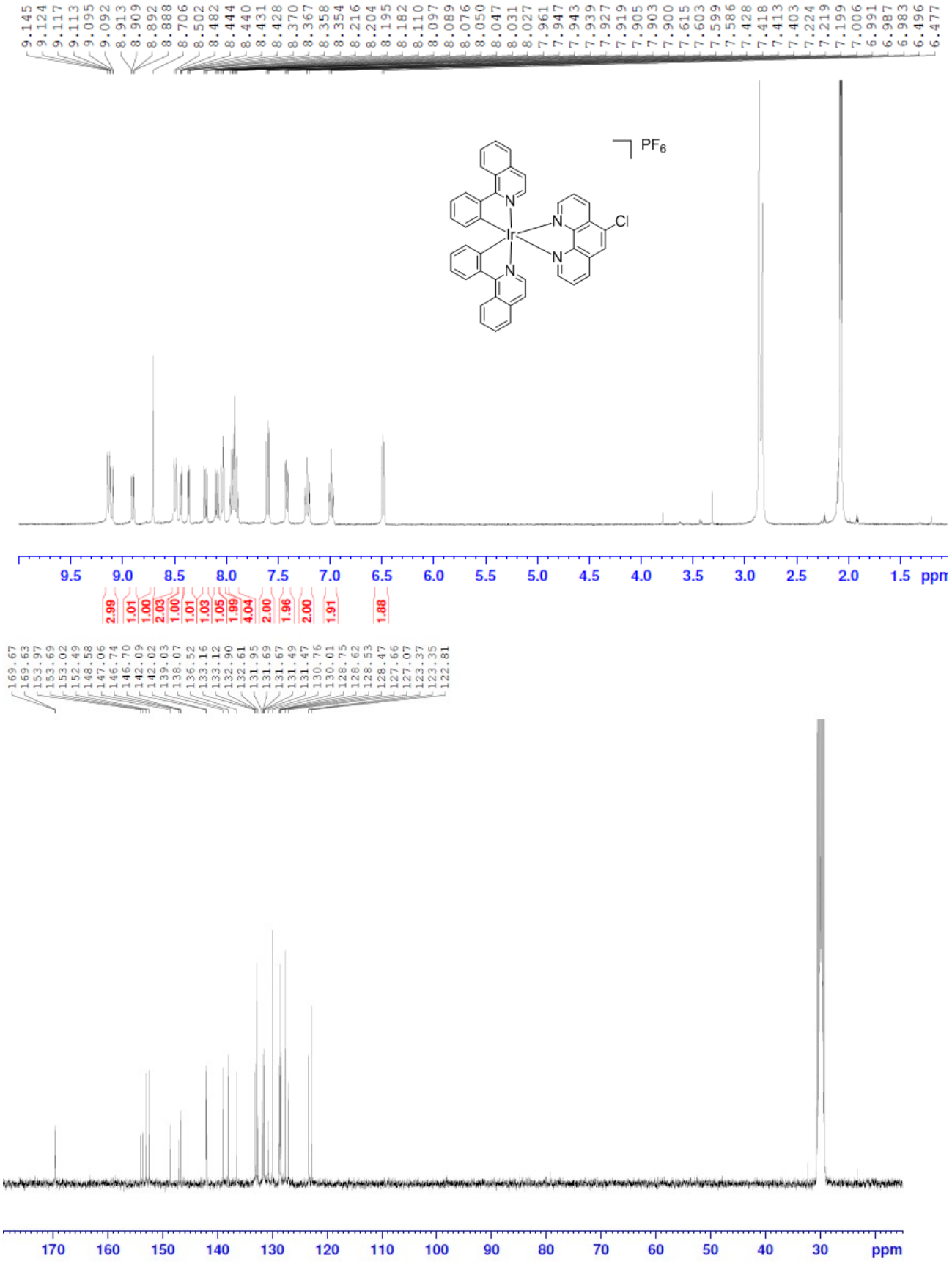
^{1}H δ 2.05, ^{13}C δ 29.8). Chemical shifts (δ) are quoted in ppm, the downfield direction being defined as positive. Uncertainties in chemical shifts are typically ±0.01 ppm for ^{1}H and ±0.05 for ^{13}C. Coupling constants are typically ±0.1 Hz for ^{1}H–^{1}H and ±0.5 Hz for ^{1}H–^{13}C couplings. The following abbreviations are used for convenience in reporting the multiplicity of NMR resonances: s, singlet; d, doublet; t, triplet; m, multiplet. All NMR data was acquired and processed using standard Bruker software (Topspin).

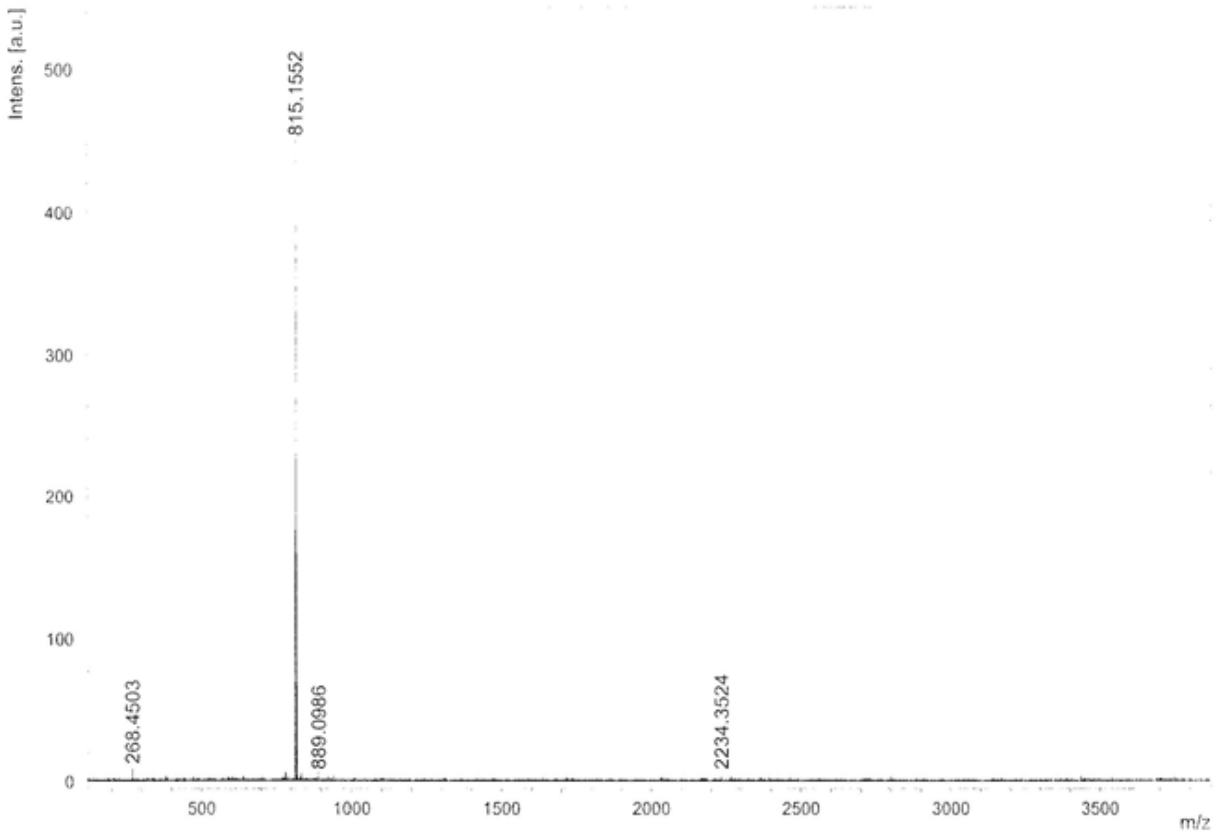
Photophysical measurement. Emission spectra and lifetime measurements for complex **1** were performed according to the previously reported method.[1]

Synthesis

The complex **1** was prepared according to (modified) literature method.[2] Specifically, a suspension of $[Ir_2(piq)_4Cl_2]$ (0.2 mmol) and corresponding N^N ligands 5-chloro-1,10-phenanthroline (Cl-phen) (0.44 mmol) in a mixture of dichloromethane:methanol (1:1, 20 mL) was refluxed overnight under a nitrogen atmosphere. The resulting solution was then allowed to cool to room temperature, and filtered to remove unreacted cyclometallated dimer. To the filtrate, a solution of ammonium hexafluorophosphate (0.5 g in 5 mL methanol) was added and the filtrate was reduced in volume by rotary evaoration until precipitation of the crude product occurred. The precipitate was then filtered and washed with several portions of water (2 × 50 mL) followed by diethyl ether (2 × 50 mL). The product was recrystallized by acetonitrile:diethyl ether vapor diffusion to yield the titled compound. The complex **1** is characterized by melting point analysis, IR, ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis. Yield: 62%; m.p.: 360-363 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 9.14–9.09 (m, 3H), 8.90 (d, J = 8.0 Hz, 1H), 8.71 (s, 1H), 8.49 (d, J = 7.2 Hz, 2H), 8.44 (d, J = 4.0 Hz, 1H), 8.36 (d, J = 8.0 Hz, 1H), 8.20 (dd, J = 4.0 Hz, 2.8 Hz, 1H), 8.09 (dd, J = 4.0 Hz, 2.8 Hz, 1H), 8.04 (d, J = 8.0 Hz, 2H), 7.96–7.90 (m, 4H), 7.60 (t, J = 8.0 Hz, 2H), 7.41 (dd, J = 4.0 Hz, 2.8 Hz, 2H), 7.22 (t, J = 4.0 Hz, 2H), 6.98 (t, J = 4.0 Hz, 2H), 6.49 (d, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, Acetone-d_6) δ169.6, 154.0, 153.7, 153.0, 152.5, 148.6, 147.0, 146.7, 142.0, 149.0, 138.0, 136.5, 133.1, 132.9, 132.6, 131.9, 131.7, 131.5, 130.7, 130.0, 128.7, 128.6, 128.5, 128.4, 127.7, 127.0, 123.4, 122.8. HRMS: Calcd. for $C_{42}H_{27}IrClN_4[M–PF_6]^+$: 815.1553 Found: 815.1552 Anal. ($C_{42}H_{27}IrClN_4PF_6 + H_2O$) C, H, N: calcd. 51.56, 2.99, 5.73; found 51.78, 2.93, 5.83;

IR(KBr): 3039(ν_{C-H}), 1616($\nu_{C=C}$ or $\nu_{C=N}$) cm^{-1}, 1575($\nu_{C=C}$ or $\nu_{C=N}$) cm^{-1}, 1538($\nu_{C=C}$ or $\nu_{C=N}$) cm^{-1}, 1421($\nu_{C=C}$ or $\nu_{C=N}$) cm^{-1}, 842(δ_{C-Cl}) cm^{-1}.

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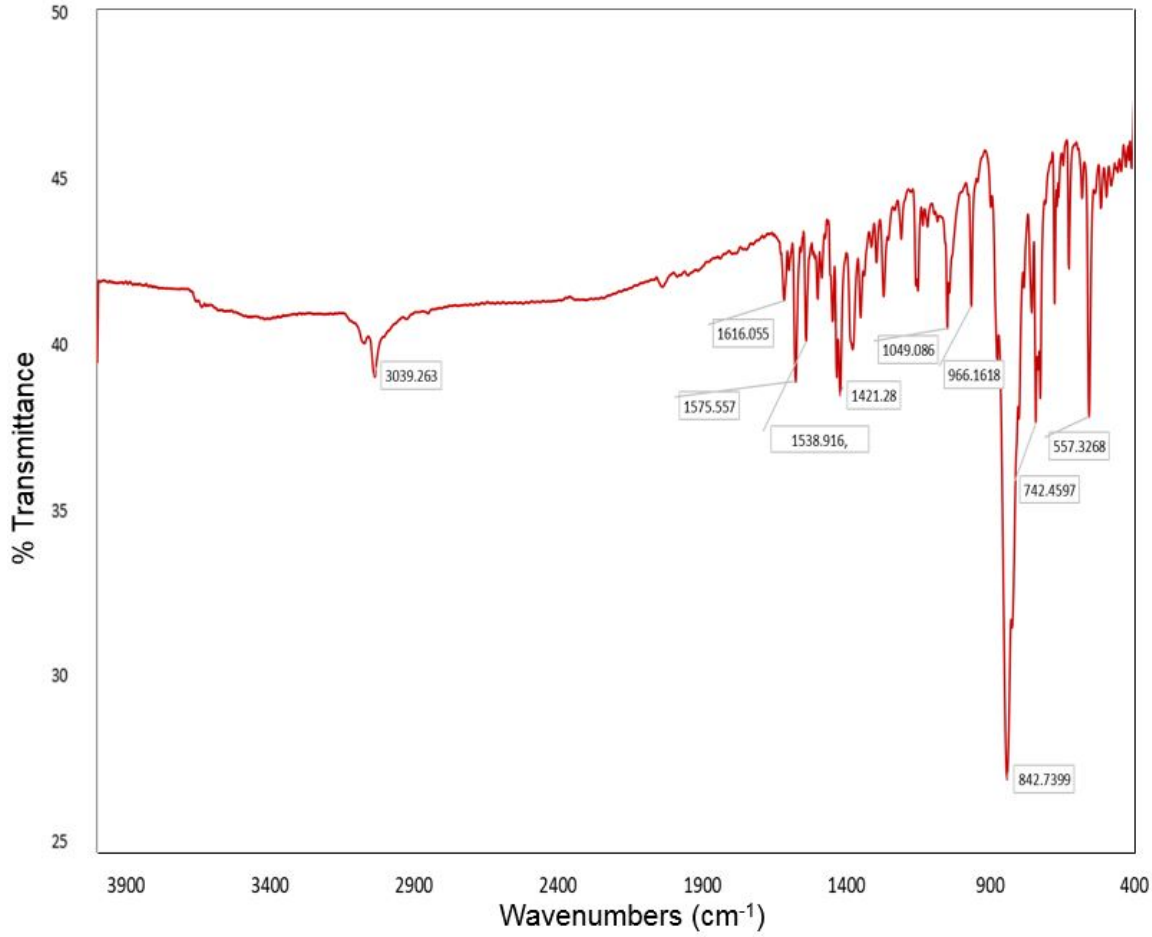
m/z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742.4597
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3900
3400
2900
2400
1900
1400
900
400
Wavenumbers (cm-1)

Complexes **2**–**5**. Reported[2,3]

Luminescence response of iridium(III) complexes towards different forms of DNA

The i-motif DNA-forming sequences (c-MYC and HIF-1α) were annealed in phosphate buffer (10 mM NaH_2PO_4, pH=5.0). G-quadruplex DNA-forming sequences (PS2.M) was annealed in Tris-HCl buffer (20 mM Tris, 50 mM KCl, pH 7.0). DsDNA was annealed in Tris-HCl buffer (20 mM Tris, pH 7.0). All of the prepared DNA were stored at –20 °C before use. Complexes **1**–**5** (0.5 μM) was added to 5 μM of ssDNA, dsDNA, i-motif DAN and G-quadruplex DNA in phosphate buffer (10 mM NaH_2PO_4, pH=5.0).

Absorption titration

A solution of complex **1** (20 μM) was prepared in Tris-HCl buffer (20 mM, pH 7.0). Aliquots of a millimolar stock solution of pre-annealed HIF-1α (0–20 μM), ds17 (0–20 μM), or ssDNA CCR5-DEL (0–20 μM) were added. Absorption spectra were recorded in the spectral range λ = 200–600 nm after equilibration at 20.0 °C for 10 min. The intrinsic binding constant, *K*, was determined from a plot of $D/\Delta\varepsilon ap$ vs *D* according to equation (**1**):[4]

$$D/\Delta\varepsilon ap = D/\Delta\varepsilon + 1/(\Delta\varepsilon \times K) \quad (\mathbf{1})$$

where *D* is the concentration of DNA, $\Delta\varepsilon ap = |\varepsilon A - \varepsilon F|$, $\varepsilon A = A_{obs}/[ligand]$, and $\Delta\varepsilon = |\varepsilon B - \varepsilon F|$; εB and εF correspond to the extinction coefficients of DNA−ligand adduct and unbound ligand, respectively.

Total cell extract preparation

The TRAMPC1 (ATCC® CRL2730™) cell line were purchased from American Type Culture Collection (Manassas, VA 20108 USA). Prostate cancer cells were trypsinized and resuspended in TE buffer (10 mM Tris-HCl 7.4, 1 mM EDTA). After incubation on ice for 10 min, the lysate was centrifuged and the supernatant was collected.

Evaluating the effect of dNTP composition

To evaluate the effect of the dNTP composition on the activity of randomly synthesized C-rich DNA sequence, the polymerase reaction were conducted as follows:

A reaction containing 1 μM DNA primer, 50 μL TdT reaction buffer (0.2 M potassium cacodylate, 0.025 M Tris, 0.01% (v/v) Triton X-100, 1 mM $CoCl_2$, pH 7.2), different compositions of 1 mM dNTP including various combinations of dCTP (percentage ranging from 50% to 100%), dATP

(percentage ranging from 0% to 50%) and dTTP (percentage ranging from 0% to 50%) and indicated concentrations of TdT, were incubated in at 37 °C for 2 h. The polymerase reaction is terminated by heating the solution at 75°C for 10 min. Subsequently, the mixture was cooled down and was diluted using phosphate buffer (10 mM NaH_2PO_4, pH=5.0) to a final volume of 500 μL, and 0.5 μM of complex **1** was added to the mixture. Emission spectra were recorded in the 550−750 nm range using an excitation wavelength of 310 nm.

Detection of TdT activity

To detect TdT activity, the experiments were carried out under the same conditions as above, except using certain composition of 1 mM dNTP (e.g. 60% dCTP and 40% dTTP) and different concentrations of TdT ranging from 0.25 U to 12 U.

For the detection of TdT activity in cell extract, the experiment was carried out under the same condition as the TdT detection in buffered solution, except the buffer containing 0.5% (v/v) cell extract.

Table S1 Photophysical properties of iridium(III) complex **1**

Complex	Quantum yield	λ_{em}/ nm	Life time/ μs	UV/vis absorption λ_{abs} / nm (ε/ $dm^3mol^{-1}cm^{-1}$)
1	0.052	604	4.698	233 (4.6×10^4), 290 (2.88×10^4), 352 (1.15×10^4), 448 (2.76×10^3)

Table S2 DNA sequences used in this project:

	Sequence
Primer	5′-GTTAACCTAGCCAG-3′
CCR5-DEL	5′-CTCAT_4C_2ATACAT_2A_3GATAGTCAT-3′
ds17	5′-$C_2AGT_2CGTAGTA_2C_3$-3′ 5′-$G_3T_2ACTACGA_2CTG_2$-3′
c-MYC	5′- CCCCACCTTCCCCACCCTCCCCACCCTCCCC -3′
HIF-1α	5′- CCCGCCCCCTCTCCCTCCCAAA -3′
PS2.M	5′-GTGGGTAGGGCGGGTTGG-3′

Fig. S1 Chemical structures of Ir(III) complexes **2**–**5** used in this study.

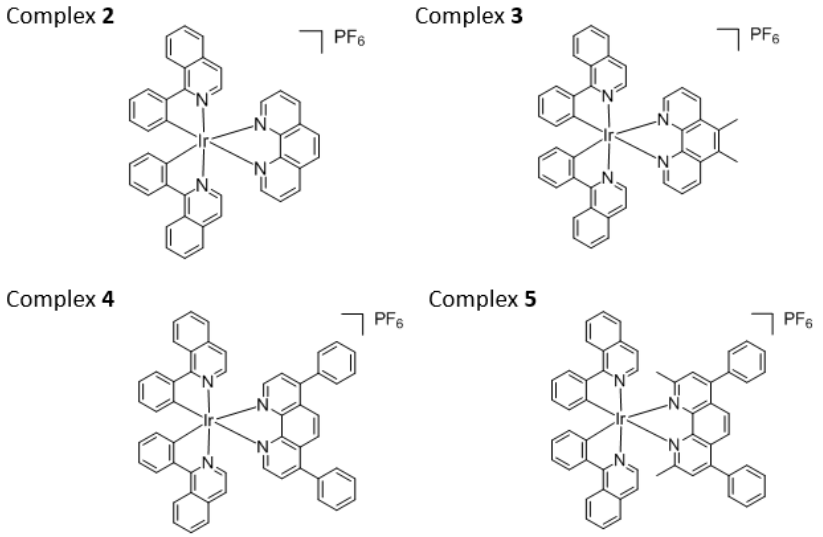


Fig. S2 Luminescence response of complexes **1**–**5** (0.5 μM) in phosphate buffer (10 mM NaH_2PO_4, pH=5.0) in the presence of 5 μM ssDNA (CCR5-DEL), 5 μM DNA (ds17) and 5 μM i-motif (HIF-1α), respectively. I-motif was pre-annealed in phosphate buffer (10 mM NaH_2PO_4, pH=5.0).

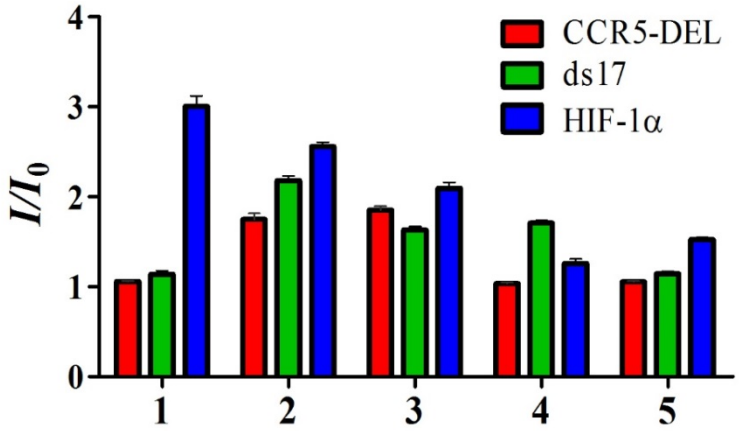


Fig. S3 Plot of D/Δεap vs. concentration of DNA for calculating the intrinsic binding constant (*K*). Absorbance of **1** at 360 nm was used for calculation. Intrinsic binding constant of **1** to HIF-1α i-motif $K = 1.53 \times 10^5$ M^{-1}; ds17 duplex DNA $K = 0.74 \times 10^5$ M^{-1}; CCR5-DEL ssDNA $K = 0.68 \times 10^5$ M^{-1} in phosphate buffer (10 mM NaH_2PO_4, pH=5.0).

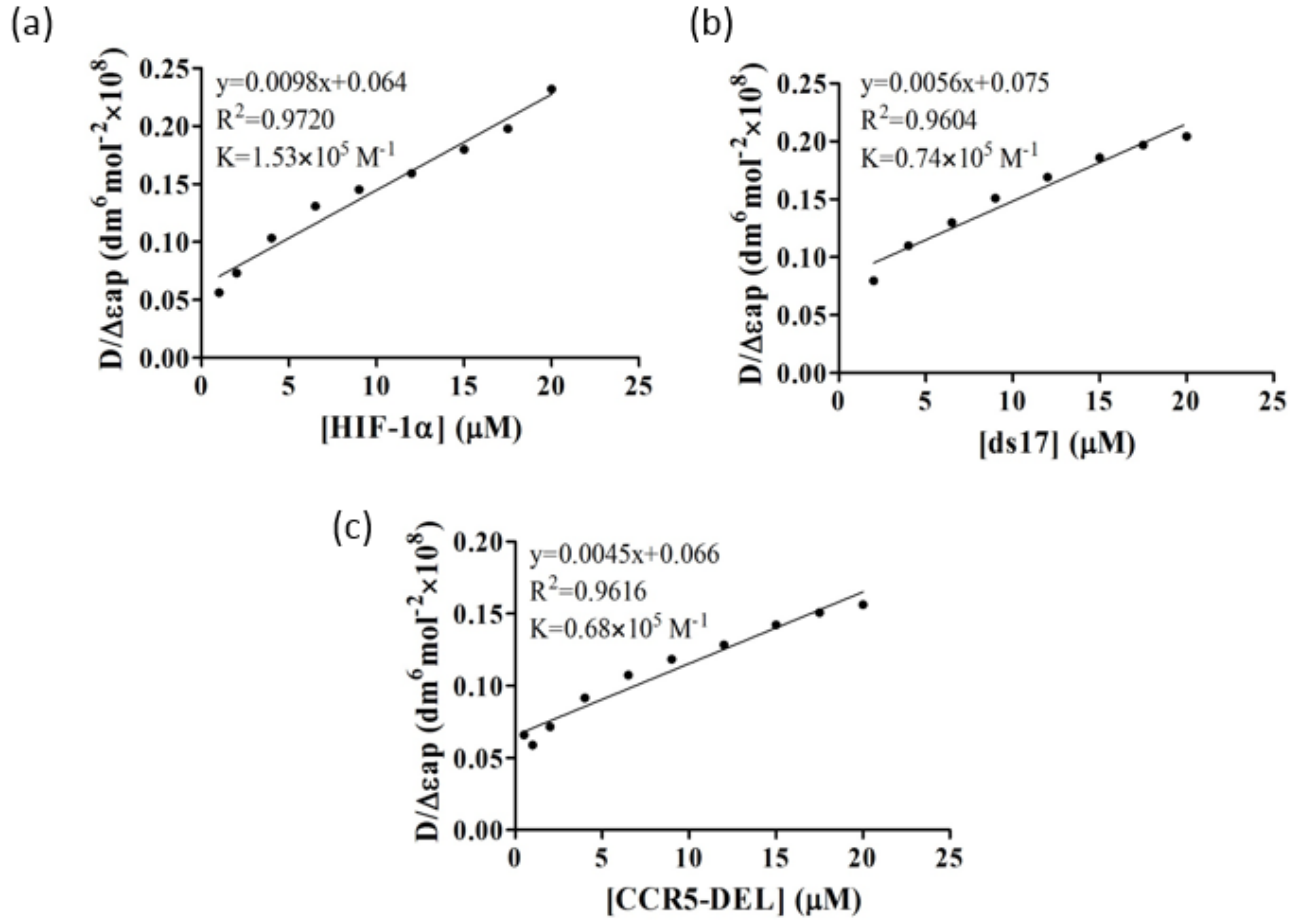


Fig. S4 Luminescence response of complex **1** (0.5 μM) to the DNA generated by dNTP pool or dCTP + dTTP pool. Experiment conditions: a mixture of 1 μM DNA primer, 50 μL TdT reaction buffer, 1 mM dNTP or 1 mM 60% dCTP + 40% dTTP and 4 U/mL TdT, were incubated 2 h at 37 °C for 2 h. The mixture was cooled down and was diluted using phosphate buffer (10 mM NaH_2PO_4, pH = 5.0) to a final volume of 500 μL, and 0.5 μM of complex **1** was added to the mixture.

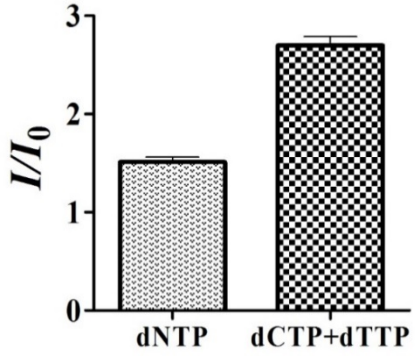


Fig. S5 Circular dichroism (CD) spectra for characterizing the DNA conformation of the TdT-synthesized DNAzyme: (a) 40 U/mL TdT; (b) C-rich DNA pool generated i-motif DNA; (c) dNTP pool generated random-coil DNA. The final concentration of primer DNA and TdT was 0.75 μM and 40 U/mL in phosphate buffer (10 mM NaH_2PO_4, pH = 5.0), respectively.

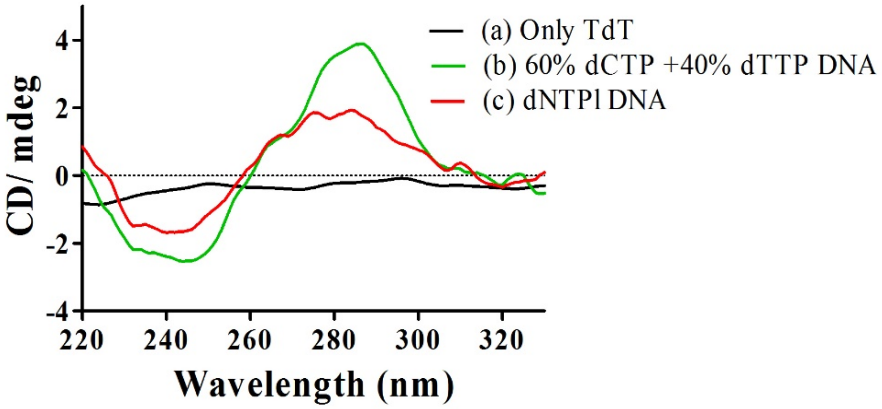


Fig. S6 Luminescence response of complex **1** (0.5 μM) to the DNA generated by the C-rich dNTP pool. Experiment conditions: 1 μM DNA primer, 50 μL TdT reaction buffer, different compositions of 1 mM dNTP including various combinations of dCTP (percentage ranging from 50% to 100%), dATP (percentage ranging from 0% to 50%) and dTTP (percentage ranging from 0% to 50%) and 4 U/mL TdT, were incubated at 37 °C for 2 h, the mixture was cooled down and diluted by phosphate buffer (10 mM NaH_2PO_4, pH = 5.0) to a final volume of 500 μL.

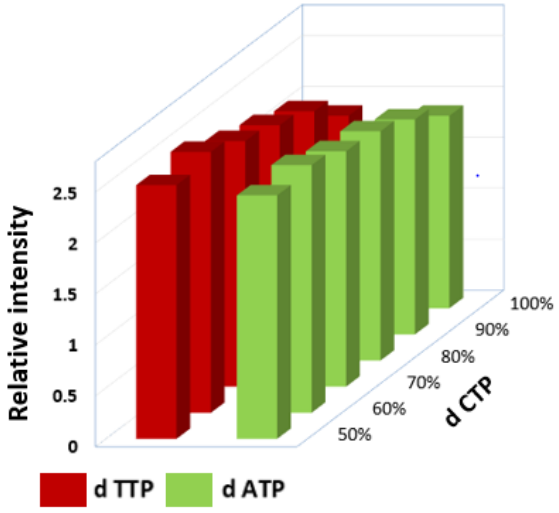


Fig. S7 Luminescence responses of complex **1** (0.5 μM) at various concentrations of primer (0, 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 μM). Experimental conditions: Various concentrations of primers in 50 μL reaction buffer, containing 1mM 60% dCTP + 40% dTTP, were treated with 4 U/mL TdT at 37 °C for 2 h, and diluted by phosphate buffer (10 mM NaH_2PO_4, pH = 5.0) to a final volume of 500 μL.

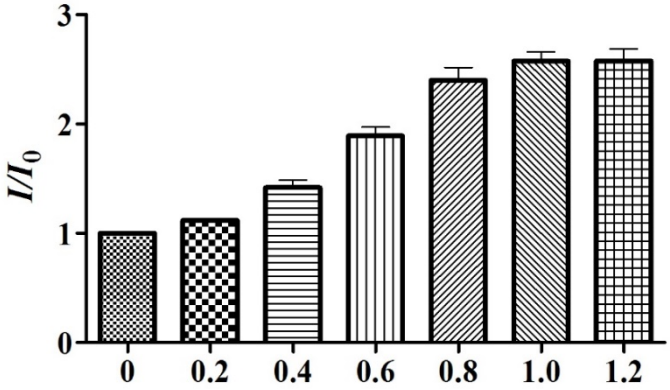


Fig. S8 Luminescence responses of different concentrations (0.25, 0.5, 0.75 and 1 μM) of complex **1**. Experimental conditions: 1 μM primer DNA in 50 μL reaction buffer containing 1 mM 60% dCTP + 40% dTTP were treated with 4 U/mL TdT at 37 °C for 2 h, and diluted by phosphate buffer (10 mM NaH_2PO_4, pH = 5.0) to a final volume of 500 μL. Subsequently, different concentrations of complex **1** were added to the mixture.

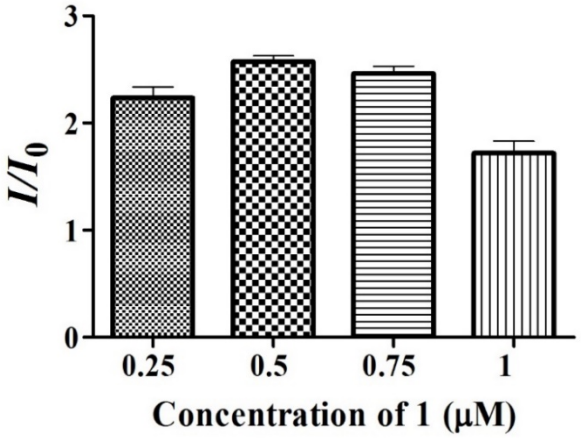


Fig. S9 Luminescence responses of complex **1** (0.5 μM) at various pH (4.0, 5.0, 6.0, 7.0 and 8.0). Experimental conditions: 1 μM primer DNA in 50 μL reaction buffer containing 1 mM 60% dCTP + 40% dTTP were treated with 4 U/mL TdT at 37 °C for 2 h, and diluted by different pH phosphate buffer (10 mM NaH_2PO_4) to a final volume of 500 μL.

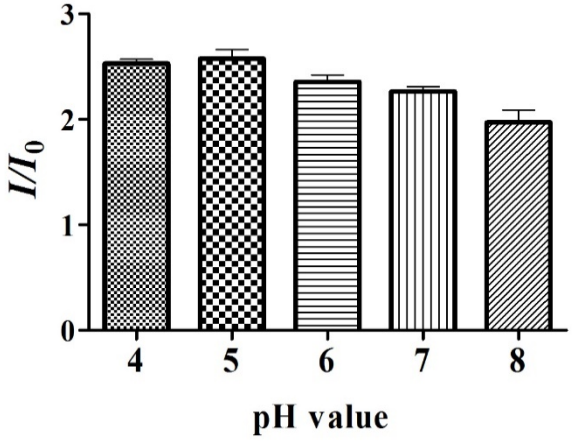


Fig. S10 Luminescence responses of complex **1** (0.5 μM) at the different reaction time. Reaction conditions: 50 μL reaction buffer containing primer DNA (1 μM) and 1mM 60% dCTP + 40% dTTP was treated with 4U/mL TdT at 37 °C with different reaction time, and diluted by phosphate buffer (10 mM NaH_2PO_4, pH = 5.0).

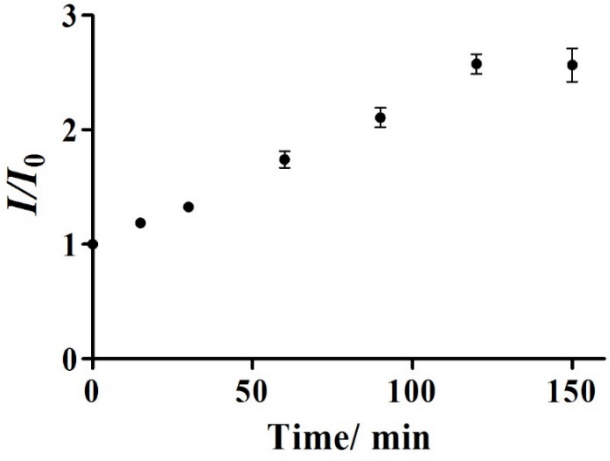


Fig. S11 Emission spectral traces of complex **1** (0.5 μM) in the 50 μL reaction buffer containing primer DNA (1 μM) and 1mM 60% dCTP + 40% dTTP was treated by TdT at 37 °C for 2 h, and diluted by phosphate buffer (10 mM NaH_2PO_4, pH = 5.0) into 500 μL showing a signal-to-noise ratio greater than 3.

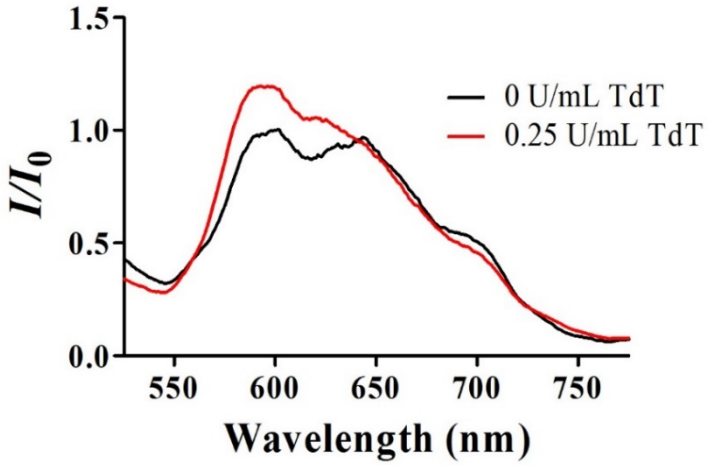


Fig. S12 Luminescence responses of complex **1** (0.5 μM) to 12 independent samples for TdT activity detection. Experimental conditions: 1 μM primer DNA in 50 μL reaction buffer were treated with 4 U/mL TdT at 37 °C for 2 h, and diluted by phosphate buffer (10 mM NaH_2PO_4, pH = 5.0) to a final volume of 500 μL.

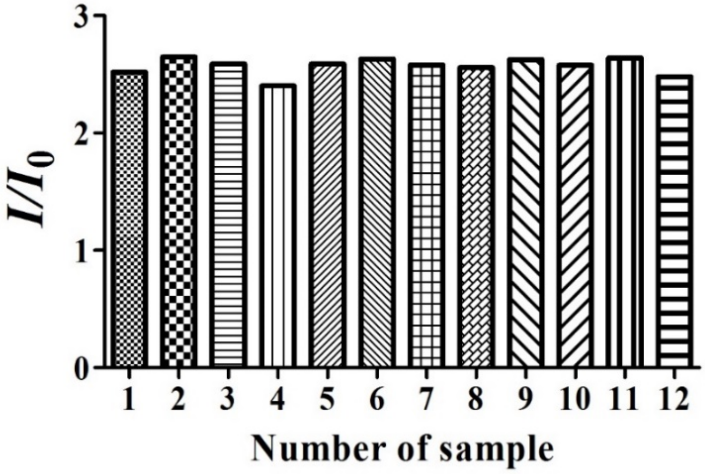
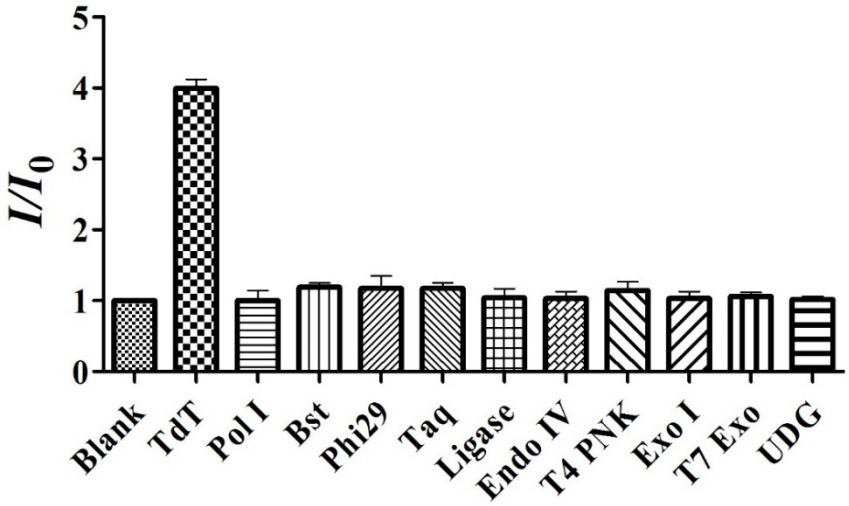


Fig. S13 Selectivity of the i-motif-based assay for TdT over other polymerases and DNA-modifying enzymes. The concentration of the enzymes was 8 U/mL. (Pol I: DNA polymerase I lg (Klenow); Bst: Bst DNA polymerase large fragment; Phi29: phi29 DNA polymerase; Taq: Taq DNA polymerase; Ligase: E. coli DNA ligase; Endo IV: endonuclease IV; T4 PNK: T4 polynucleotide kinase; Exo I: exonuclease I; T7 exonuclease; UDG: uracil-DNA glycosylase).



References

1. C. Yang, L. M. Fu, Y. Wang, J. P. Zhang, W. T. Wong, X. C. Ai, Y. F. Qiao, B. S. Zou and L. L. Gui, *Angew. Chem.*, 2004, **116**, 5120-5123.
2. Q. Zhao, S. Liu, M. Shi, C. Wang, M. Yu, L. Li, F. Li, T. Yi and C. Huang, *Inorg. Chem.*, 2006, **45**, 6152-6160.
3. L. Lu, D. S.-H. Chan, D. W. Kwong, H.-Z. He, C.-H. Leung and D.-L. Ma, *Chem. Sci.*, 2014, **5**, 4561-4568.
4. C. Kumar and E. H. Asuncion, *J. Am. Chem. Soc.*, 1993, **115**, 8547-8553.