Supporting Information

Iridium(III) Complexes with 1,10-Phenanthroline-based N^N Ligands as Highly Selective Luminescent G-quadruplex Probes and Application for Switch-on Ribonuclease H Detection

Lihua Lu,*[a,b] Wanhe Wang,[b] Chao Yang,[c] Tian-Shu Kang,[c] Chung-Hang Leung*[c] and Dik-Lung Ma*[b]

[a] College of Chemistry and Pharmaceutical Sciences, Qingdao Agricultural University, Qingdao 266109, China

[b] Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China

[c] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China

* Corresponding author:

Dr. Lihua Lu, Email: lulihua2012@hotmail.com, Tel: (+86) 13954297004

Dr. Dik-Lung Ma, E-mail: edmondma@hkbu.edu.hk, Fax: (+852) 3411-7348, Tel: (+852) 3411-7075.

Dr. Chung-Hang Leung, E-mail: duncanleung@umac.mo Tel: (+853) 8822-4688

Materials

RNA was dissolved in DEPC water to a concentration of 100 μM. 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). RNase H was purchased from New England Biolabs Inc. (Beverly, MA, USA).

General experimental

Mass spectrometry was performed at the Mass Spectroscopy Unit at the Department of Chemistry, Hong Kong Baptist University, Hong Kong (China). Melting points were determined using a Gallenkamp melting apparatus and are uncorrected. Deuterated solvents for NMR purposes were obtained from Armar and used as received.

^{1}H and ^{13}C NMR were recorded on a Bruker Avance 400 spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). ^{1}H and ^{13}C chemical shifts were referenced internally to solvent shift (acetone-d_6: ^{1}H, 2.05, ^{13}C, 29.8). Chemical shifts (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; q, quartet; m, multiplet. All NMR data was acquired and processed using standard Bruker software (Topspin).

Circular dichroism (CD) spectra were recorded on a JASCO-815 spectropolarimeter using 1 cm path length quartz cuvettes. Spectra was collected between 220 nm and 335 nm, using 2 cm bandwidth, 50 nm min^{-1} scan speed and two scans. The data were baseline corrected using CD spectra of buffer alone.

Photophysical measurement

Emission spectra and lifetime measurements for complexes **1**–**7** were performed on a PTI TimeMaster C720 Spectrometer (Nitrogen laser: pulse output 337 nm) fitted with a 455 nm filter. Error limits were estimated: λ (±1 nm); τ (±10%); φ (±10%). All solvents used for the lifetime measurements were degassed using three cycles of freeze-vacuum-thaw.

Luminescence quantum yields were determined using the method of Demas and Crosby [1] $[Ru(bpy)_3][PF_6]_2$ in degassed acetonitrile as a standard reference solution ($\Phi_r = 0.0602$) and calculated according to the reported equation:

$$\Phi_s = \Phi_r(B_r/B_s)(n_s/n_r)^2(D_s/D_r)$$

where the subscripts s and r refer to sample and reference standard solution respectively, n is the refractive index of the solvents, D is the integrated intensity, and Φ is the luminescence quantum yield. The quantity B was calculated by $B = 1 - 10^{-AL}$, where A is the absorbance at the excitation wavelength and L is the optical path length.

Luminescence responses of complexes 1–7 towards different forms of DNA.

Stock solutions of complexes **1**–**7** were obtained by dissolving the complexes in acetonitrile to a concentration of 1 mM. The G-quadruplex DNA-forming sequences Pu22, c-kit1, c-kit87up, dim-G4, HTS, Pu27 and PS2.M were annealed in Tris-HCl buffer (10 mM Tris, 75 mM KCl, pH 7.4) and were stored at –20 °C before use. Complexes **1**–**7** (1 μM) was added to 5 μM of ssDNA, dsDNA or various G-quadruplex DNA in Tris-HCl buffer (10 mM Tris-HCl, pH 7.4), then their emission intensity were tested.

The luminescence selectivity ratio ($I_{G\text{-quadruplex}}/I_{ssDNA}$ or $I_{G\text{-quadruplex}}/I_{dsDNA}$) of complexes **1**–**7** (Figure S1) is defined as the luminescence response enhancement of the complexes towards G-quadruplex ($I_{G\text{-quadruplex}}/I_0$) divided by the luminescent response enhancement towards ssDNA (I_{ssDNA}/I_0) or dsDNA (I_{dsDNA}/I_0), respectively.

FRET melting assay.

The ability of **7** to stabilize G-quadruplex DNA 5′-FAM-$GTG_3TAG_3CG_3T_2$ G_2-TAMRA-3′ (F-PS2.M-T) or dsDNA 5′-FAM-TATAGCTA-HEG-TATAGCTATAT-

TAMRA-3′ (F-10-T) was investigated using a fluorescence resonance energy transfer (FRET) melting assay. The experimental procedure was similar to previously described.[2]

G-quadruplex fluorescent intercalator displacement (G4-FID) assay.

The G4-FID experiment was to evaluate the binding affinity of **7** to G-quadruplex DNA PS2.M or dsDNA ds17. The experiment procedure was the same as previously reported.[3]

Total cell extract preparation

The TRAMP-C1 (ATCC® CRL2730™) cell line was 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.

Detection of RNase H in buffered solution.

The G4 DNA PS2.M (100 μM) and the designed RNA (100 μM) were mixed in an equimolar ratio and heated to 95 °C for 10 min, and then cooled down gradually to 25 °C over 2 h to obtain a stable double-stranded RNA/DNA hybrid, which was verified by CD spectroscopy. The prepared RNA/DNA complex was stored at –20 °C before use. For RNase H detection, certain amount of RNA/DNA hybrid (50 μM) was incubated with various amounts of RNase H in RNase H reaction buffer (50 mM Tris-HCl, 75 mM KCl, 3 mM $MgCl_2$, 10 mM DTT, pH 8.3 @ 25°C) at 37 °C in a total reaction of volume of 50 μL. The mixture was incubated for 50 min to allow the digestion of the RNA portion of the RNA/DNA hybrid and the release of the G-rich DNA sequence. RNase H was then inactivated by heating the mixture to 95 °C for 5 min, followed by the addition of K^+ ions (final concentration 50 mM) to promote the formation of the G-quadruplex structure. Finally, the reaction solution was diluted to 500 μL with Tris-HCl buffer (10 mM Tris-HCl, pH 7.4), and 0.75 μM complex **7** was added to the mixture. Emission spectra were recorded in the 480−760 nm range using an excitation wavelength of 310 nm. The detection of RNase H in cell extract was

carried out according to the operation procedures used in the buffered solution. The only difference is that the RNase H reaction buffer was added into 5 μL cell extract.

Synthesis

Complexes **1** and **2**: Reported.[4]

Complex **3**: Reported.[5]

Complex **4**: Reported.[6]

Complexes **5** and **6**: Reported.[4]

Complex **7**: The complex **7** was synthesized according to a modified literature method. The precursor iridium(III) complex dimer $[Ir_2(phq)_4Cl_2]$ (2-phenylquinoline) was prepared as previously reported.[7] Then, a suspension of $[Ir_2(phq)_4Cl_2]$ (0.1 mmol) and the corresponding N^N ligand 4,7-dimethyl-1,10-phenanthroline) (0.22 mmol) in a mixture of DCM:methanol (1:1.2, 20 mL) was refluxed overnight under a nitrogen atmosphere. The work-up procedure was the same as previously reported [8]. Complex **7** was characterized by ^{1}H-NMR, ^{13}C-NMR, high resolution mass spectrometry (HRMS) and elemental analysis.

Complex **7**. Yield: 40%. ^{1}H NMR (400 MHz, acetone-d_6) δ 8.59–8.51 (m, 4H), 8.47 (d, J = 9.0 Hz, 2H), 8.29 (d, J = 8.0 Hz, 2H), 8.23 (s, 2H), 7.91 (d, J = 5.4 Hz, 2H), 7.81 (dd, J = 8.1 Hz, 2H), 7.40–7.33 (m, 2H), 7.28 (t, J = 8.0 Hz, 2H), 7.21 (d, J = 8.1 Hz, 2H), 6.94–6.81 (m, 4H), 6.66 (dd, J = 7.8 Hz, 2H), 2.89 (s, 6H). ^{13}C NMR (100 MHz, acetone-d_6) δ 171.32, 152.36, 149.89, 148.98, 148.51, 147.29, 147.11, 141.01, 135.57, 131.47, 131.33, 131.05, 129.97, 128.55, 128.29, 128.22, 127.49, 125.15, 125.11, 123.71, 118.91, 18.99. MALDI-TOF-HRMS: Calcd. for $C_{44}H_{32}IrN_4$ $[M–PF_6]^+$: 809.2256 found: 809.2244. Anal.: ($C_{44}H_{32}F_6IrN_4P$ + $3H_2O$) C, H, N: calcd. 52.43, 3.80, 5.56; found 52.21, 3.40, 5.67.

Table S1. Photophysical properties of iridium(III) complexes **1**–**7** in acetonitrile at 298 K.

Complex	Quantum yield	λ_{ex}/ nm	λ_{em}/ nm	Lifetime /μs	UV/vis absorption λ_{abs} / nm (ε/ $dm^3mol^{-1}cm^{-1}$)
1	0.12	297	570	8.13	270 (5.72×10^4), 333 (2.06×10^4)
2	0.06	295	575	1.84	234 (2.55×10^4), 262 (2.20×10^4), 286 (2.67×10^4), 350 (7.91×10^3)
3	0.38	292	566	4.84	228 (6.7 × 103), 282 (1.2 × 104)
4	0.26	289	560	3.43	214 (9.717×10^4), 240 (6.091×10^4), 282 (7.874×10^4), 337 (2.896×10^4)
5	0.09	293	570	1.96	270 (3.13×10^4), 337 (2.33×10^4)
6	0.27	297	583	4.31	280 (3.6×10^4), 429 (5.9×10^3)
7	0.24	290	565	4.27	238 (6.72×10^4), 274 (7.99×10^4), 338 (2.83×10^4), 444 (7.86×10^3)

Table S2. DNA sequences used in this project:

DNA	Sequence
PS2.M	5′- $GTG_3TAG_3CG_3T_2G_2$-3′
RNA	5′-CCAACCCGCCCUACCCAC-3′
$PS2.M_m$	5′-GT*GTG*TAG*T*GCG*T*GTTG*C*-3′
RNA_m	5′-*G*CAAC*A*CGC*A*CUA*CAC*AC-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-kit87up	5′- $AG_3AG_3CGCTG_3AG_2AG_3$ -3′
HTS	5′- $G_3T_2AG_3T_2AG_3T_2AG_3$-3′
Pu22	5′- $G_2T_2G_2TGTG_2T_2G_2$-3′
dim-G4	5′- $G_3T_3G_3T_4G_3T_4G_3$-3′
Pu27	5′- $TG_4AG_3TG_4AG_3TG_4A_2G_2$-3′
c-kit1	5′-$AG_3AG_3CGCTG_3AGGAG_3$-3′
F-PS2.M-T	5′-FAM-($GTG_3TAG_3CG_3T_2G_2$)-TAMRA-3′
F-10-T	5′-FAM-TATAGCTA-HEG-TATAGCTATAT-TAMRA-3′

The underline italic bases are mutated bases.

Table S3 DNA sequences used in loop effect experiments:

	n	Sequence
	1	5′-GTG$_3$AG$_3$CG$_3$T$_2$G$_2$-3′
	2	5′-GTG$_3$TAG$_3$CG$_3$T$_2$G$_2$-3′
	3	5′-GTG$_3$TATG$_3$CG$_3$T$_2$G$_2$-3′
	4	5′-GTG$_3$T$_2$ATG$_3$CG$_3$T$_2$G$_2$-3′
5′-side	5	5′-GTG$_3$T$_2$AT$_2$G$_3$CG$_3$T$_2$G$_2$-3′
loop	6	5′-GTG$_3$T$_3$AT$_2$G$_3$CG$_3$T$_2$G$_2$-3′
	7	5′-GTG$_3$T$_3$AT$_3$G$_3$CG$_3$T$_2$G$_2$-3′
	9	5′-GTG$_3$T$_4$AT$_4$G$_3$CG$_3$T$_2$G$_2$-3′
	11	5′-GTG$_3$T$_5$AT$_5$G$_3$CG$_3$T$_2$G$_2$-3′
	13	5′-GTG$_3$T$_6$AT$_6$G$_3$CG$_3$T$_2$G$_2$-3′
	15	5′-GTG$_3$T$_7$AT$_7$G$_3$CG$_3$T$_2$G$_2$-3′
	17	5′-GTG$_3$T$_8$AT$_8$G$_3$CG$_3$T$_2$G$_2$-3′
	1	5′-GTG$_3$TAG$_3$CG$_3$T$_2$G$_2$-3′
	2	5′- GTG$_3$TAG$_3$TCG$_3$T$_2$G$_2$-3′
	3	5′- GTG$_3$TAG$_3$TCTG$_3$T$_2$G$_2$-3′
	4	5′- GTG$_3$TAG$_3$T$_2$CTG$_3$T$_2$G$_2$-3′
central	5	5′- GTG$_3$TAG$_3$T$_2$CT$_2$G$_3$T$_2$G$_2$-3′
loop	6	5′-GTG$_3$TAG$_3$T$_3$CT$_2$G$_3$T$_2$G$_2$-3′
	7	5′-GTG$_3$TAG$_3$T$_3$CT$_3$G$_3$T$_2$G$_2$-3′
	9	5′-GTG$_3$TAG$_3$T$_4$CT$_4$G$_3$T$_2$G$_2$-3′
	11	5′-GTG$_3$TAG$_3$T$_5$CT$_5$G$_3$T$_2$G$_2$-3′
	13	5′-GTG$_3$TAG$_3$T$_6$CT$_6$G$_3$T$_2$G$_2$-3′
	15	5′-GTG$_3$TAG$_3$T$_7$CT$_7$G$_3$T$_2$G$_2$-3′
	17	5′-GTG$_3$TAG$_3$T$_8$CT$_8$G$_3$T$_2$G$_2$-3′
	1	5′-GTG$_3$TAG$_3$CG$_3$TG$_2$-3′
	2	5′-GTG$_3$TAG$_3$TCG$_3$T$_2$G$_2$-3′
	3	5′-GTG$_3$TAG$_3$TCG$_3$T$_3$G$_2$-3′
	4	5′-GTG$_3$TAG$_3$TCG$_3$T$_4$G$_2$-3′
3′-side	5	5′-GTG$_3$TAG$_3$TCG$_3$T$_5$G$_2$-3′
loop	6	5′-GTG$_3$TAG$_3$TCG$_3$T$_6$G$_2$-3′
	7	5′-GTG$_3$TAG$_3$TCG$_3$T$_7$G$_2$-3′

9	5′-$GTG_3TAG_3TCG_3T_9G_2$-3′
11	5′-$GTG_3TAG_3TCG_3T_{11}G_2$-3′
13	5′-$GTG_3TAG_3TCG_3T_{13}G_2$-3′
15	5′-$GTG_3TAG_3TCG_3T_{15}G_2$-3′
17	5′-$GTG_3TAG_3TCG_3T_{17}G_2$-3′

Table S4 Comparison of the fluorescent or colorimetric-based RNase H activity assays reported in recent years.

Method	Detection limit	Reference	Labeled DNA?
A Series of Iridium(III) Complexes With 2-Phenylquinoline Ligands as Highly Selective Luminescent Switch-on G-quadruplex Probes And an Application in Ribonuclease H Detection	0.125 U/mL	This work	No
A Quadruplex-Based, Label-Free, and Real-Time Fluorescence Assay for RNase H Activity and Inhibition	0.2 U/mL	[9]	No
Colorimetric Detection of HIV-1 Ribonuclease H Activity by Gold Nanoparticles	27 U/mL	[10]	No

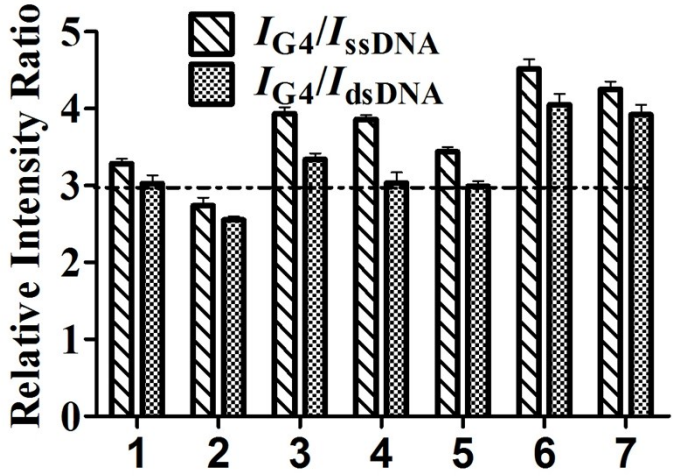

Figure S1. Diagrammatic bar array representation of the luminescence enhancement ratio of complexes **1**–**7** for PS2.M G4 DNA over ssDNA (CCR5-DEL) or dsDNA (ds17).

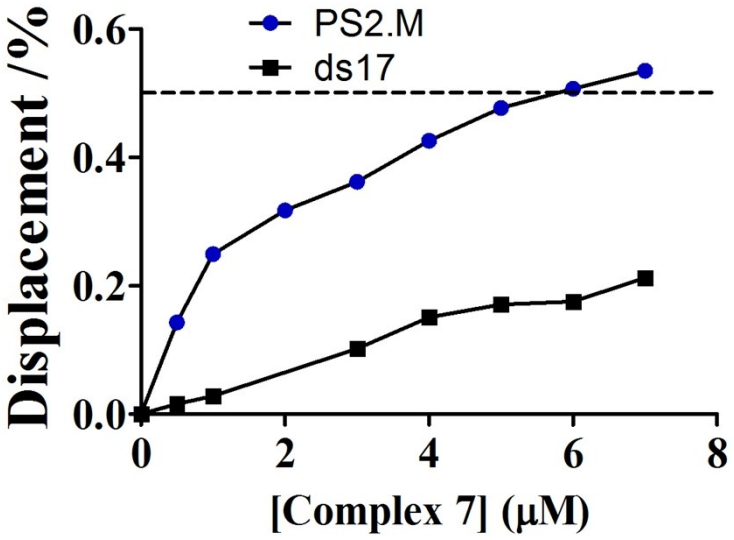

Figure S2. G4-FID profiles for G4 DNA PS2.M and duplex DNA ds17 with the increasing concentration of **7** in Tris-HCl buffer.

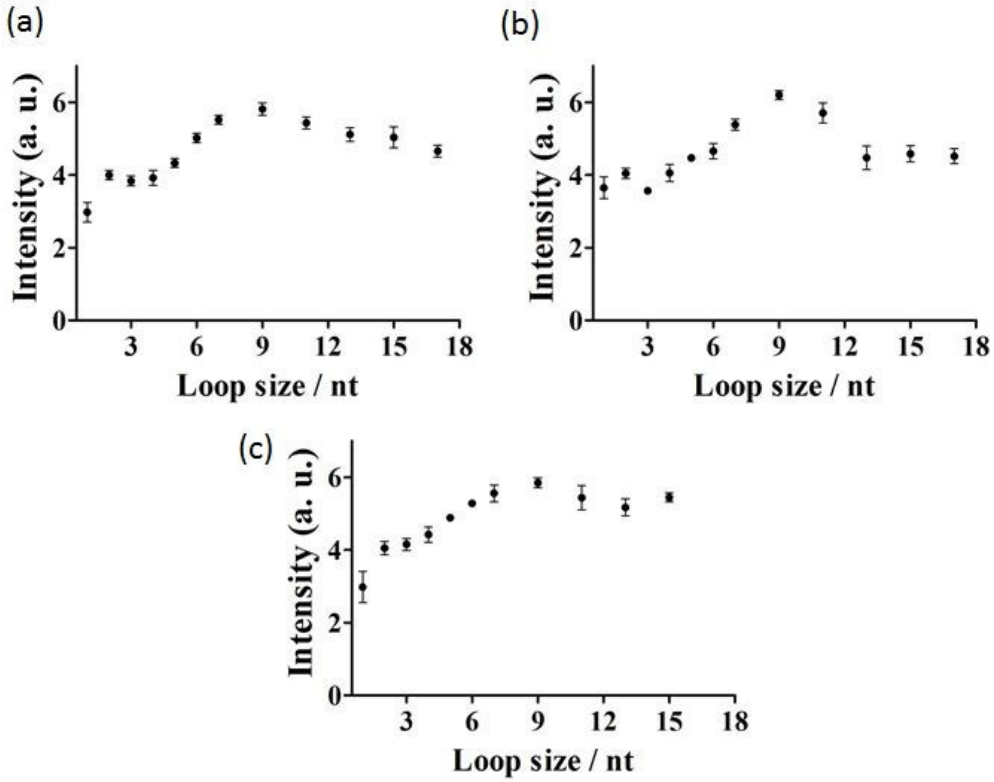

Figure S3. (a) Luminescence enhancement of complex **7** as a function of loop size (a) 5′-side loop: 5′-GTG$_3$**T$_x$AT$_y$**G$_3$CG$_3$T$_2$G$_2$-3′ (n = 1 + x + y = 1–7, 9, 11, 13, 15 and 17) (b) central loop: 5′-GTG$_3$TAG$_3$**T$_x$CT$_y$**G$_3$T$_2$G$_2$-3′ (n = 1 + x + y = 1–7, 9, 11, 13, 15 and 17) and (c) 3′-side loop 5′- GTG$_3$TAG$_3$CG$_3$**T$_n$**G$_2$-3′ (n = 1–7, 9, 11, 13, 15 and 17) (in nucleotides, the bold parts are the loop parts of PS2.M. The specific sequences are in Table S3).

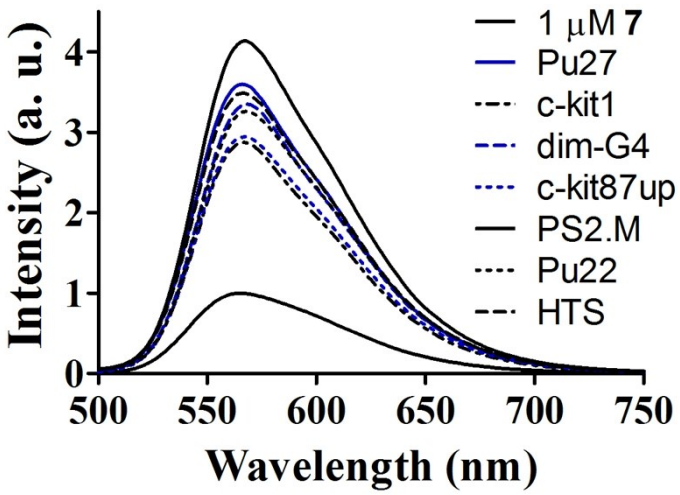

Figure S4. The luminescence responses of complex **7** in the presence of different G4 DNA.

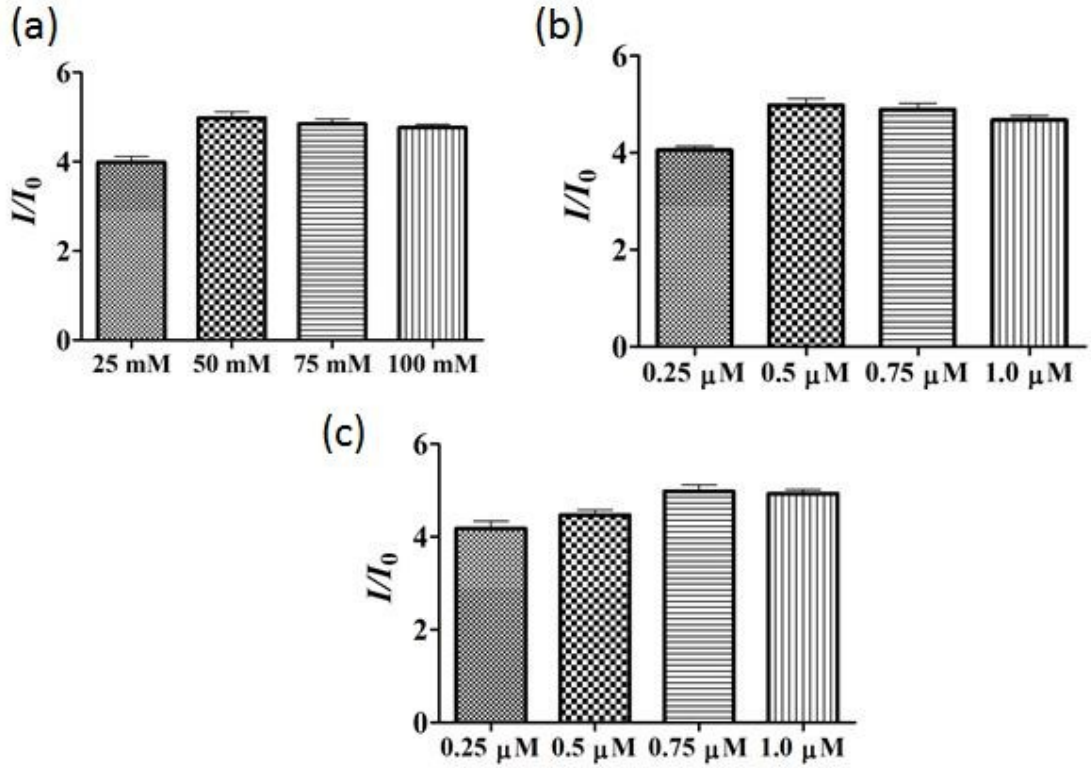

Figure S5. Relative luminescence enhancement of the reaction system in the presence or absence of 4 U/mL of RNase H (a) at different concentrations (25, 50, 75 and 100 mM) of K^+, (b) at different concentrations (0.25, 0.5, 0.75 and 1.0 μM) of RNA/DNA hybrid, (c) at different concentrations (0.25, 0.5, 0.75 and 1.0 μM) of complex **7**.

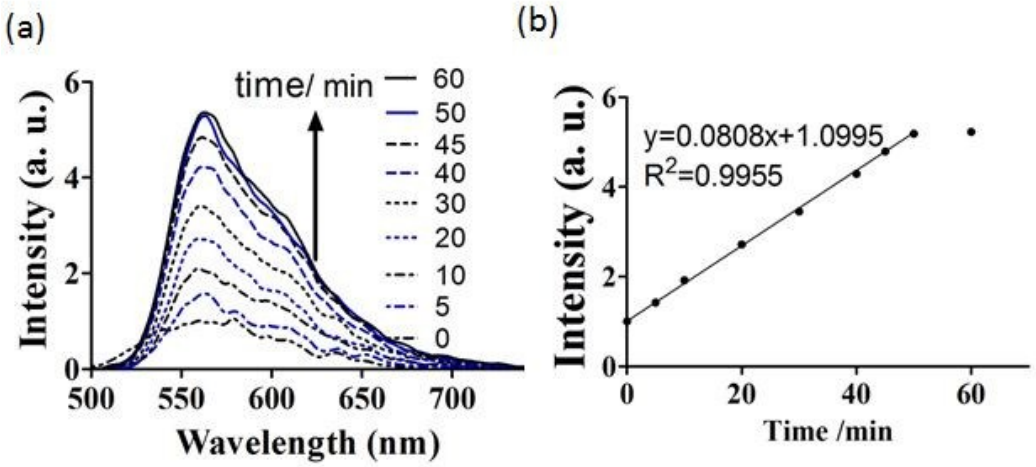

Figure S6. (a) Luminescence responses of the reaction system at the different reaction time, (b) the linear relation of signal enhancement of the reaction system with different reaction time.

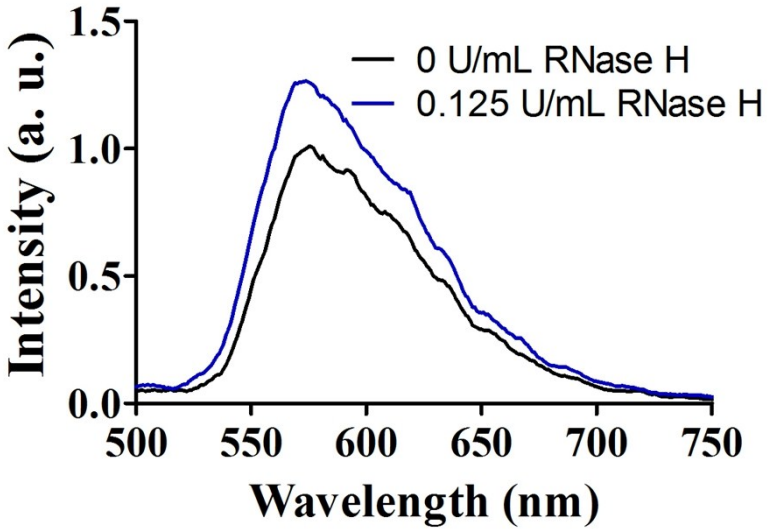

Figure S7. Emission spectral traces of complex **7** (0.75 μM), in the absence of or presence of RNase H (0.125 U/mL) in Tris-HCl buffer (10 mM Tris-HCl, 50 mM KCl, pH 7.4).

Reference

[1] G.A. Crosby, J.N. Demas, Measurement of photoluminescence quantum yields. Review, J. Phys. Chem., 75 (1971) 991-1024.

[2] P. Yang, A. De Cian, M.P. Teulade-Fichou, J.L. Mergny, D. Monchaud, Engineering Bisquinolinium/Thiazole Orange Conjugates for Fluorescent Sensing of G-Quadruplex DNA, Angew. Chem. Int. Edit, 121 (2009) 2222-2225.

[3] D. Monchaud, C. Allain, H. Bertrand, N. Smargiasso, F. Rosu, V. Gabelica, A. De Cian, J.-L. Mergny, M.-P. Teulade-Fichou, Ligands playing musical chairs with G-quadruplex DNA: a rapid and simple displacement assay for identifying selective G-quadruplex binders, Biochimie, 90 (2008) 1207-1223.

[4] K.-H. Leung, H.-Z. He, B. He, H.-J. Zhong, S. Lin, Y.-T. Wang, D.-L. Ma, C.-H. Leung, Label-free luminescence switch-on detection of hepatitis C virus NS3 helicase activity using a G-quadruplex-selective probe, Chemical Science, 6 (2015) 2166-2171.

[5] M. Wang, K.-H. Leung, S. Lin, D.S.-H. Chan, C.-H. Leung, D.-L. Ma, A G-quadruplex-based, label-free, switch-on luminescent detection assay for Ag+ ions based on the exonuclease III-mediated digestion of C–Ag+–C DNA, Journal of Materials Chemistry B, 2 (2014) 6467-6471.

[6] M. Wang, B. He, L. Lu, C.-H. Leung, J.-L. Mergny, D.-L. Ma, Label-free luminescent detection of LMP1 gene deletion using an intermolecular G-quadruplex-based switch-on probe, Biosensors and Bioelectronics, 70 (2015) 338-344.

[7] Q. Zhao, S. Liu, M. Shi, C. Wang, M. Yu, L. Li, F. Li, T. Yi, C. Huang, Series of new cationic iridium (III) complexes with tunable emission wavelength and excited state properties: structures, theoretical calculations, and photophysical and electrochemical properties, Inorg. Chem., 45 (2006) 6152-6160.

[8] L. Lu, D.S.-H. Chan, D.W. Kwong, H.-Z. He, C.-H. Leung, D.-L. Ma, Detection of nicking endonuclease activity using a G-quadruplex-selective luminescent switch-on probe, Chem. Sci., 5 (2014) 4561-4568.

[9] D. Hu, F. Pu, Z. Huang, J. Ren, X. Qu, A Quadruplex-Based, Label-Free, and Real-Time Fluorescence Assay for RNase H Activity and Inhibition, Chemistry–A European Journal, 16 (2010) 2605-2610.

[10] X. Xie, W. Xu, T. Li, X. Liu, Colorimetric Detection of HIV-1 Ribonuclease H Activity by Gold Nanoparticles, Small, 7 (2011) 1393-1396.