Electronic Supplementary Material (ESI) for Chemical Communications


# Supporting Information

# Visual specific luminescent probing of hybrid G-quadruplex DNA by a ruthenium polypyridyl complex

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## Experimental Procedures

### Materials

All of the oligonucleotides were purchased from Sangon Biotech (Shanghai, China) and used without further purification. Samples are prepared in different buffers as described in the references: buffer A ($Na^+$ buffer: 100 mM NaCl, 10 mM $NaH_2PO_4/Na_2HPO_4$, 1 mM $Na_2EDTA$, pH = 7.0), buffer B ($K^+$ buffer: 100 mM KCl, 10 mM $NaH_2PO_4/Na_2HPO_4$, 1 mM $Na_2EDTA$, pH = 7.0) and buffer C (100 mM KCl, 10 mM $NaH_2PO_4/Na_2HPO_4$, 1 mM $Na_2EDTA$, pH = 5.5).

**Table S1**. DNA sequence introduced in the experiment

| Squences | Abb. | Expected conformations | Ref. |
|---|---|---|---|
| 5'-AGGGTTAGGGTTAGGGTTAGGG-3' | $AG_3(T_2AG_3)_3$ | antiparallel/mix | 1 |
| FAM-5'-AGGGTTAGGGTTAGGGTTAGGG-3'-TRAMA | F22T | | |
| 5'-TGGGGTTGGGGTTGGGGTTGGGGT-3' | $d(TG_4T)_4$ | parallel | 2 |
| 5'-CCCTAACCCTAACCCTAACCCT-3' | $(C_3TA_2)_3C_3T$ | i-motif | 3 |
| 5'-AAAAAAAAAAAAA-3' | polyd(A) | single strand | |
| 5'-TTTTTTTTTTTTT-3' | polyd(T) | single strand | |
| 5'-AAAAAAAAAAAAA-3' and 2*5'-TTTTTTTTTTTTT-3' | polyd(A) ·2polyd(T) | triplex | 4 |
| 5'-ATATATATATAT-3' and the complementary strand | poly(AT) | duplex | |
| 5'-GCGCGCGCGCGC-3' and the complementary strand | poly(GC) | duplex | |
| CT-DNA | CT-DNA | duplex | |

**General Methods**. Elemental analyses (C, H, and N) were carried out on a Vario EL elemental analyzer. $^1H$ NMR spectra were recorded on a Varian INOVA-500 spectrometer with $(CD_3)_2SO$ as solvent at room temperature and tetramethylsilane (TMS) as the internal standard. All chemical shifts are given relative to TMS. Electrospray mass spectra (ES-MS) were recorded on a LCQ system (Finnigan MAT, USA) and the quoted *m/z* values are for the major peaks in the isotope distribution.

**Luminescent detection**. Ru(II) complex (50 $\mu$M) and DNAs (20 $\mu$M) were mixed and

after 4 h for equilibrium, luminescent spectra were detected by Perkin Elmer LS55 Spectrofluorometer from 500 to 800 nm, the excitation wavelength was 415 nm.

**Circular Dichroic experiment.** CD spectra were measured using a JASCO J-810 spectropolarimeter, The samples of DNAs were diluted to 10 $\mu$M and placed in a 1 cm path length quartz cell, then different concentrations of the Ru(II) complex were added. CD spectra were then baseline-corrected and recorded from 200 to 400 nm.

**FRET melting assay.** This was performed with a Roche Lightcycler 2.0 real-time fluorescent quantitative PCR machine. In a 25 $\mu$L volume system, 200 nM DNA F22T was added together with different concentrations of the Ru(II) complex. The experiments were performed in the machine following the procedure of 37°C for 2 min, then an increase speed of 1°C every minute from 37 to 95°C. During the procedure we measured the FAM after each step.

**Visualization experiment.** 50 $\mu$M of the Ru(II) complex was mixed with 20 $\mu$M DNAs and incubated for 4 h. The total volume of each sample was 600 $\mu$L. Then all the samples were photographed under irradiation of UV light.

**Dialyze assay.** 50 $\mu$L different DNAs (20 $\mu$M) were added into 3500 MWCO Thermo Slide-A-Lyzer® MINI Dialysis Units, then placed into a 1 $\mu$M Ru(II) complex environment. After 6 h of equilibration, the units were removed and 10% SDS was added until it reached a concentration of 1%. Agilent Technologies 7700 Series ICP-MS was introduced to quantify the amount of Ru(II) complex in the units as well as dialyzate. The bound complex can be calculated as the difference of the two concentrations.

**Fluorescence polarization anisotropy**. The experiment was performed as described in reference[5]. 20 $\mu$M of the Ru(II) complex was mixed with 320 $\mu$M DNAs and incubated for 4 h. The total volume of every sample was 800 $\mu$L. Then the samples were excited at 415 nm and the fluorescence signal was monitored at 600 nm through crossed polarizers.

**Synthesis of Ru(II) complex**

Compounds $[Ru(bpy)_2(1,10\text{-phenanthroline-5,6-diamine})]^{2+}$ [6] and 3,4-benzo[a]phenazine quinone[7] were prepared as described previously.

**Synthesis of $[Ru(bpy)_2(bqdppz)](ClO_4)_2$ (1).**
A mixture of $[Ru(bpy)_2(1,10\text{-phenanthroline-5,6-diamine})]^{2+}$ (0.411 g, 0.5 mmol) and 3,4-benzo[a]phenazine quinone (0.130 g, 0.5 mmol) was suspended in 30 mL of acetic acid, the solution was then heated at 135 °C for 4 h under Ar. It was cooled to room temperature, and 50 $cm^3$ water were added. After filtration, a dark red precipitate was obtained by dropwise addition of aqueous $NaClO_4$ solution. The product was purified by column chromatography on alumina with acetonitrile-toluene (1:1 v/v) as eluent. Yeild: 62%. Anal. Calc. $C_{48}H_{30}Cl_2N_{10}O_8Ru$: C, 55.07; H, 2.89; N, 13.38%. Found: C,

54.93; H, 3.03; N, 13.18%. ES–MS [$CH_3CN$, m/z]: 423.9 ([M-2$ClO_4$]$^{2+}$). $^1$H NMR (500 MHz, $d_6$-DMSO): $\delta$ 9.92 (d, J = 8 Hz, 1H), 9.77 (d, J = 8 Hz, 1H), 9.51 (d, J = 6 Hz, 1H), 9.29 (d, J = 6 Hz, 1H), 8.93 – 8.87 (m, 4H), 8.52 (t, J = 5 Hz, 1H), 8.41 (t, J = 5 Hz, 1H), 8.33 (t, J = 5 Hz, 2H), 8.27 (t, J = 5 Hz, 2H), 8.17 – 8.05 (m, 8H), 7.90 (d, J = 6 Hz, 3H), 7.82 (d, J = 5 Hz, 1H), 7.65 (t, J = 4.5 Hz, 2H), 7.43 (dd, $J_1$ = 5 Hz, $J_2$ = 3 Hz, 2H).

## ES MS Spectrum

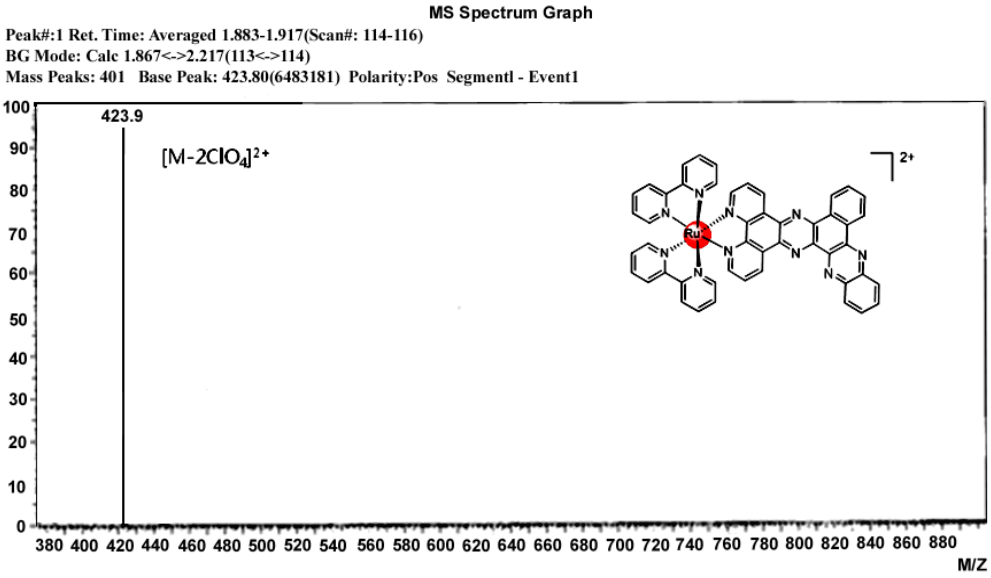


**Fig. S1** ES-MS Spectrum of complex **1**

## $^1$H NMR Spectrum

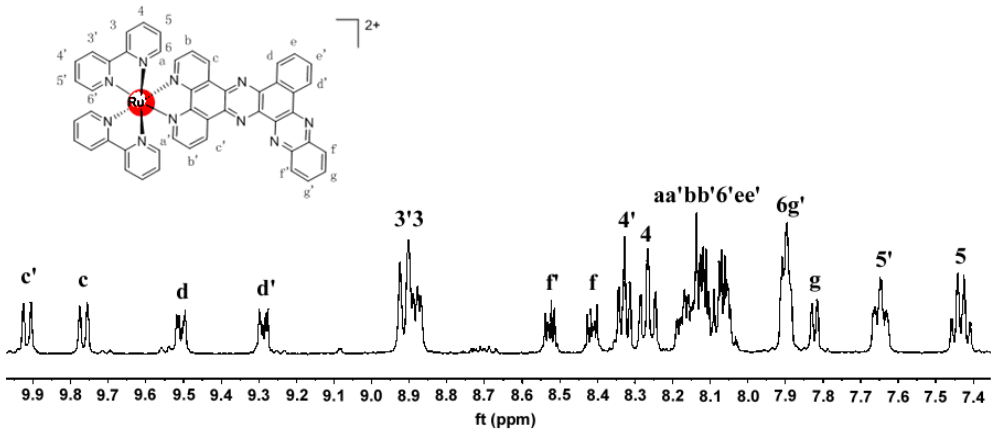


**Fig. S2** $^1$H NMR Spectrum of complex **1** in DMSO-$d_6$

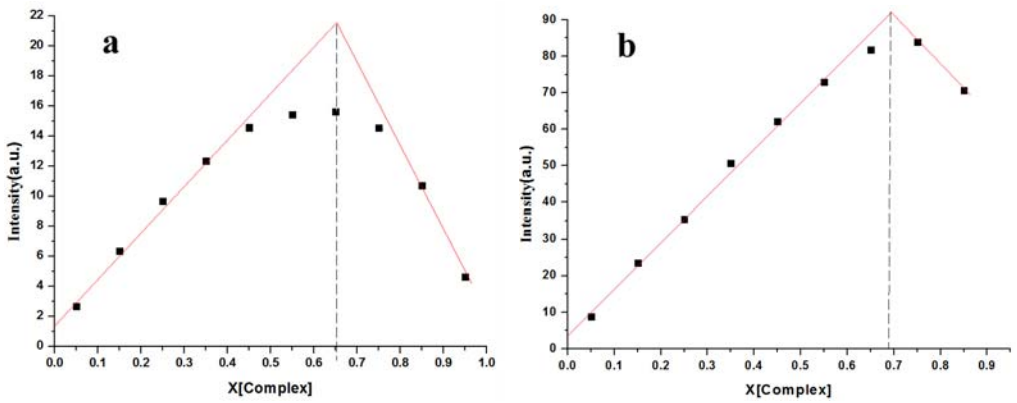


**Fig. S3** Job-Plot using luminescence data for complex **1** with $AG_3(T_2AG_3)_3$ in $Na^+$ buffer (a) and $K^+$ buffer (b), X = mole fraction of Ru(II) complex added to DNA, and the total concentration was maintained at 10 $\mu$M.

The result shows that binding stoichiometries of complex **1** with G-quadruplex is 2:1. We propose that complex **1** interacts with G-quadruplex through a stacking mode, which means the molecules stack on the top and bottom G-tetrad of the quadruplex.

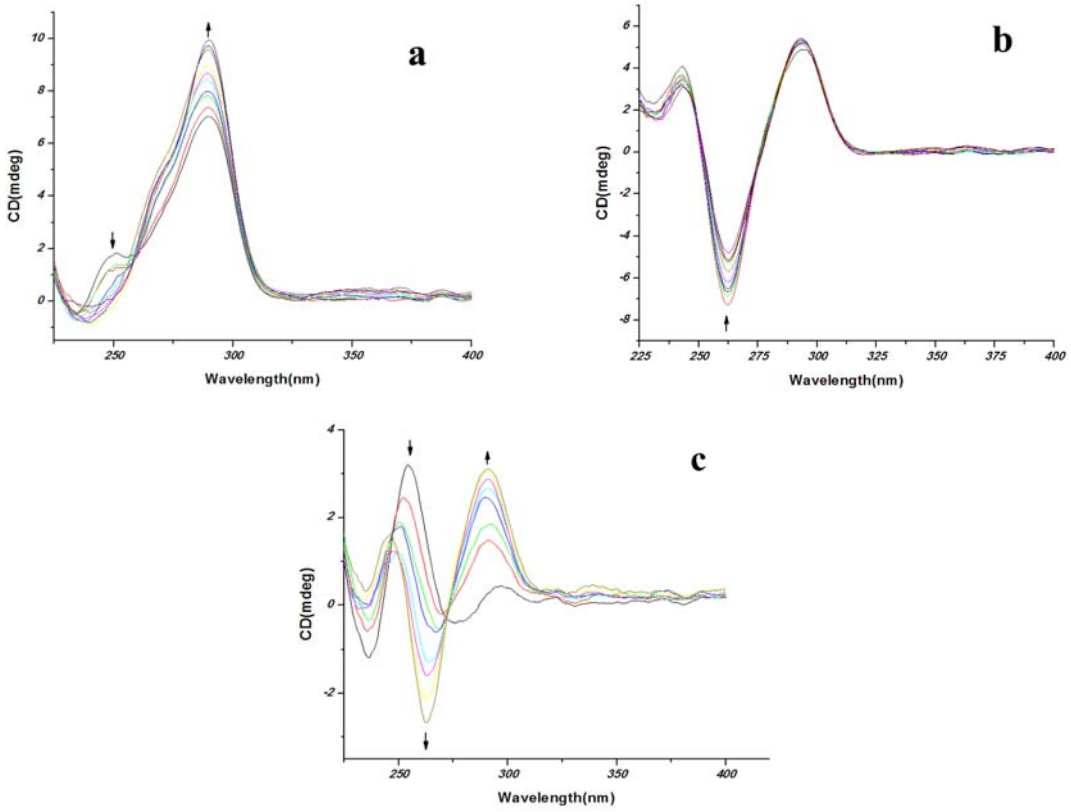


**Fig. S4** CD spectra of $AG_3(T_2AG_3)_3$ in $K^+$ buffer (a), $Na^+$ buffer (b) and $H_2O$ (c) upon increasing addition of complex. Arrows indicate the increasing concentrations of complex. The concentration of G-quadruplex was 10 $\mu$M.

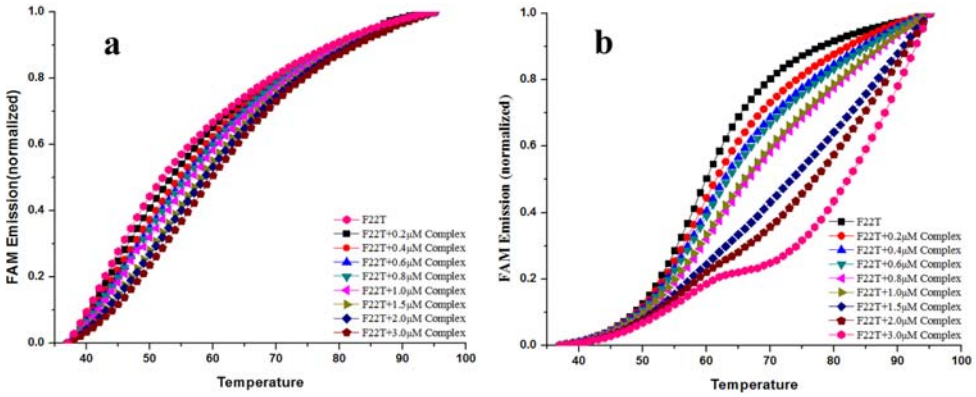


**Fig. S5** FRET melting curves detected with G-quadruplex DNA F22T (sequence: FAM-$AG_3(T_2AG_3)_3$-TRAMA, mimicking the human telomeric repeat) alone and different concentrations of complex **1** (0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 3.0 $\mu$M) in $Na^+$ buffer (a), $K^+$ buffer (b), the concentration of G-quadruplex is 200 nM.

## Reference

1. K. N. Luu, A. T. Phan, V. Kuryavyi, L. Lacroix and D. J. Patel, *J. Am. Chem. Soc.,* 2006, **128,** 9963.
2. I. N. Rujan, J. C. Meleney and P. H. Bolton, *Nucleic Acids Res.,* 2005, **33,** 2022.
3. X. Li, Y. H. Peng, J. S. Ren and X. G. Qu, *Proc. Natl. Acad. Sci. U. S. A.,* 2006, **103,** 19658.
4. P. A. Rachwal, T. Brown and K. R. Fox, *Biochemistry*, 2007, **46,** 3036.
5. C. V. Kumar, J. K. Barton and N. J. Turro, *J. Am. Chem. Soc.,* 1985, **107,** 5518.
6. S. Bodige and F. M. MacDonnell, *Tetrahedron Lett.*, 1997, **38,** 8159.
7. B. M. Zeglis and J. K. Barton, *Nature Protocols*, 2007, **2**, 357.