Electronic Supplementary Material (ESI) for Dalton Transactions
This journal is © The Royal Society of Chemistry 2013

Electronic supplementary information

$[Ru(bpy)_2dppz\text{-}idzo]^{2+}$: A colorimetric molecular "light switch" and powerful stabilizer for G-quadruplex DNA

Junliang Yao, Xing Gao, Wenliang Sun, Shuo Shi* and Tianming Yao*

1. Absorption titration with 22AG

Absorption spectra titrations were performed to determine the binding affinity between 22AG and Ru-complexes. Initially, 500 μL solutions of the blank buffer and the ruthenium complex sample (10 μM) were placed in the reference and sample cuvettes (1.0 cm path length), respectively, and then first spectrum was recorded in the range of 275-580 nm. During the titration, aliquot (5 μL) of buffered DNA (100 μM) solution was added to each cuvette to eliminate the absorbance of DNA itself, and the solutions were mixed by thorough inversion. After the solutions were mixed for ~5 min, the absorption spectra were recorded. The titration processes were repeated until there was no change in the spectra for at least four titrations, indicating binding saturation had been achieved. The changes in the Ru complex concentration due to dilution at the end of each titration were negligible. The results are shown in Fig. S1and Fig. S2.

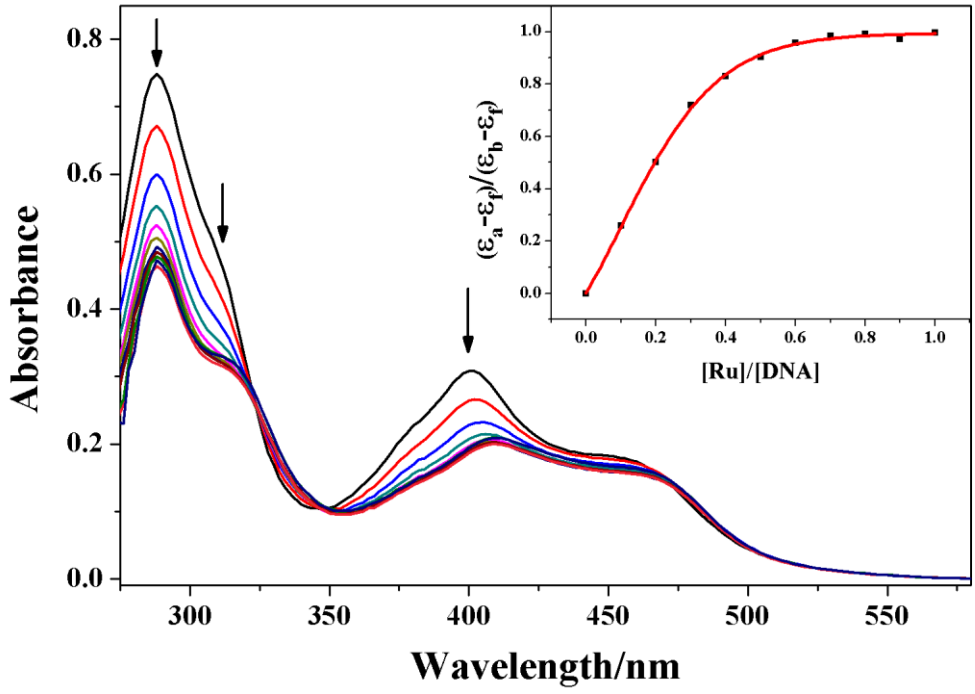

Fig. S1 Absorption spectra of $[Ru(bpy)_2dppz\text{-}idzo]^{2+}$ (10 μM) in the presence of 22AG (0–10 μM) in K^+ buffer (10 mM Tris, 100 mM KCl, pH 7.0). Inset: plot of $(\varepsilon_a-\varepsilon_f)/(\varepsilon_b-\varepsilon_f)$ *vs.* [DNA]. (using absorbance at 400 nm. ε_a, ε_f and ε_b are, the apparent extinction coefficient (A_{abs}/[M]), the extinction coefficient for free metal (M) complex and the extinction coefficient for the metal (M) complex in the fully bound form, respectively.)

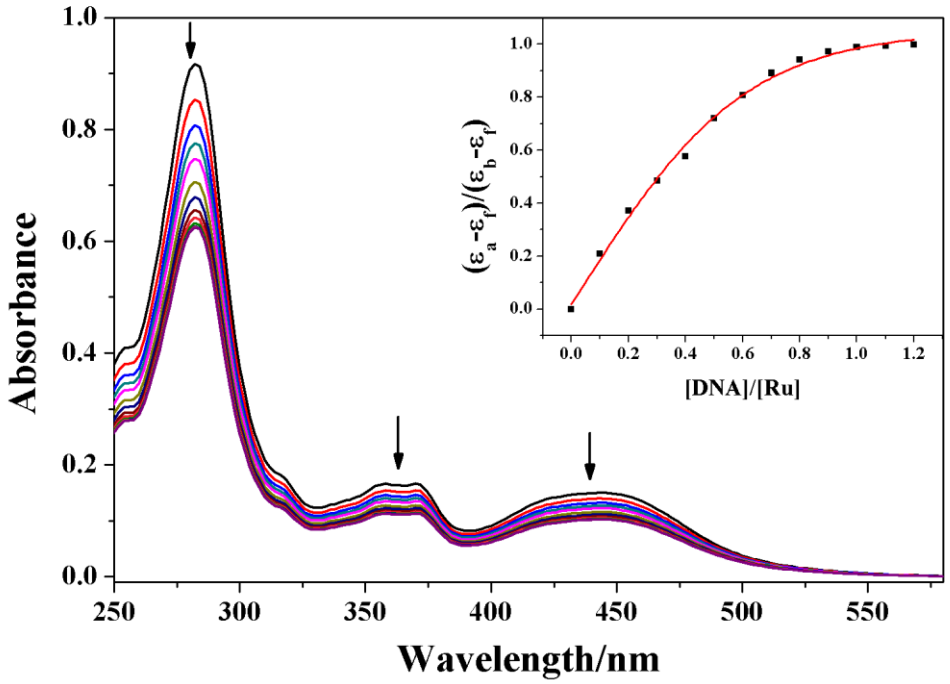

Fig. S2 Absorption spectra of $[Ru(bpy)_2dppz]^{2+}$ (10 μM) in the presence of 22AG (0–12 μM) in K^+ buffer (10 mM Tris, 100 mM KCl, pH 7.0). Inset: plot of $(\varepsilon_a-\varepsilon_f)/(\varepsilon_b-\varepsilon_f)$ *vs.* [DNA] (using absorbance at 450 nm).

The intrinsic binding constants K of Ru(II) complexes for DNA was determined using Eq. 1,[1, 2]

$$(\varepsilon_a - \varepsilon_f)/(\varepsilon_b - \varepsilon_f) = (b - (b^2 - 2K^2C_t\,[DNA]/s)^{1/2})/2KC_t \quad \text{Eq. 1a}$$

$$b = 1 + KC_t + K[DNA]/2s \quad \text{Eq. 1b}$$

where ε_a is the extinction coefficient observed for the MLCT absorption band at a given DNA concentration, ε_f is the extinction coefficient of the complex free in solution, ε_b is the extinction coefficient of the complex when fully bound to DNA. K is the equilibrium binding constant, C_t is the total metal complex concentration, $[DNA]$ is the DNA concentration in nucleotides, and s is the binding site size. The binding constants of $[Ru(bpy)_2dppz\text{-}idzo]^{2+}$ and $[Ru(bpy)_2dppz]^{2+}$ for hybrid quadruplex DNA were calculated to be 3.17×10^6 and 1.69×10^6, respectively. The values of binding constant were comparable to that obtained from fluorescence titrations.

2. Fluorescence titrations with ds-DNA

In order to clarify the difference in fluorescence behavior of our title complex towards G-quadruplex and duplex DNA, fluorescence titrations of ds-DNA (Calf Thymus DNA, CT-DNA) was also investigated on a Hitachi F-7000 fluorescence spectrophotometer. Fluorescence titration procedure: A 2 mL of 2.5 μM $[Ru(bpy)_2(dppz\text{-}idzo)]^{2+}$ in a 1.0 cm path length quartz cuvette was loaded into the fluorimeter sample block. After 5 min to allow the sample to equilibrate, the first spectrum was recorded, and then an increasing amount of 500 μM CT-DNA solution was added (1.25 μM to 25 μM, which corresponds to 0.5 to 10 equiv.) to the sample cell, followed by thorough mixing. After 5 min, the spectrum was taken again. The titration processes were repeated until there was no apparent change in the spectra for at least four times, indicating the achievement of binding saturation.

This journal is © The Royal Society of Chemistry 2013

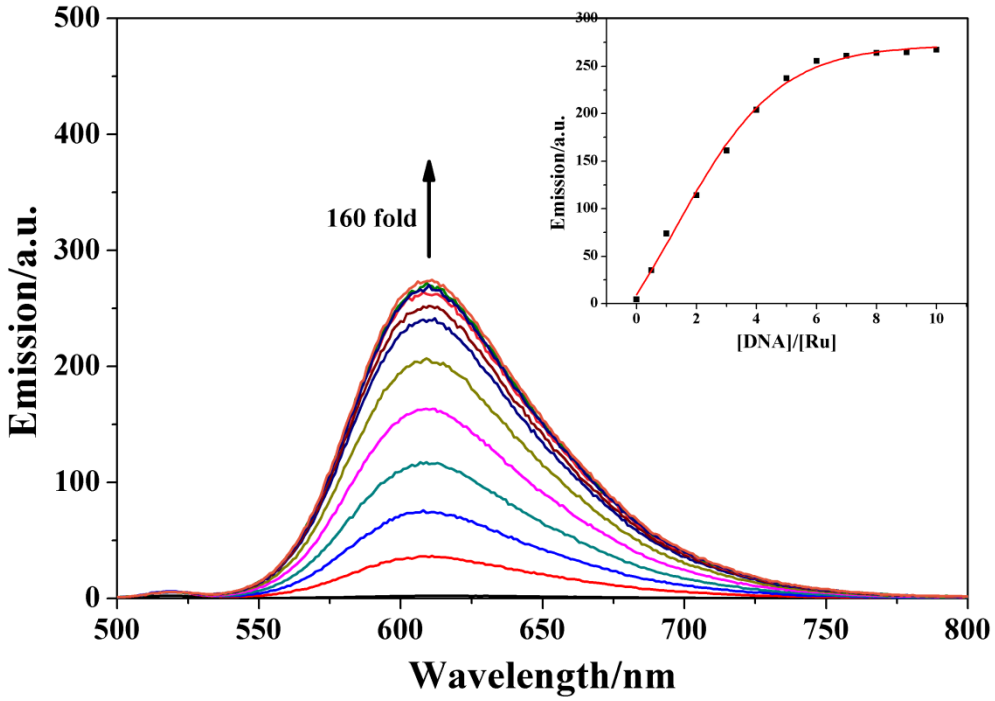

Fig. S3 Fluorescence spectra of $[Ru(bpy)_2dppz\text{-}idzo]^{2+}$ (2.5 μM) upon titration with calf thymus DNA (CT-DNA) in buffer solution containing 10 mM Tris, 50 mM NaCl, 1 mM EDTA, pH 7.5. Inset: plot of emission intensity at 605 nm *vs.* [DNA]/[Ru] and the best fit for the titration of Ru(II) complex with CT-DNA.

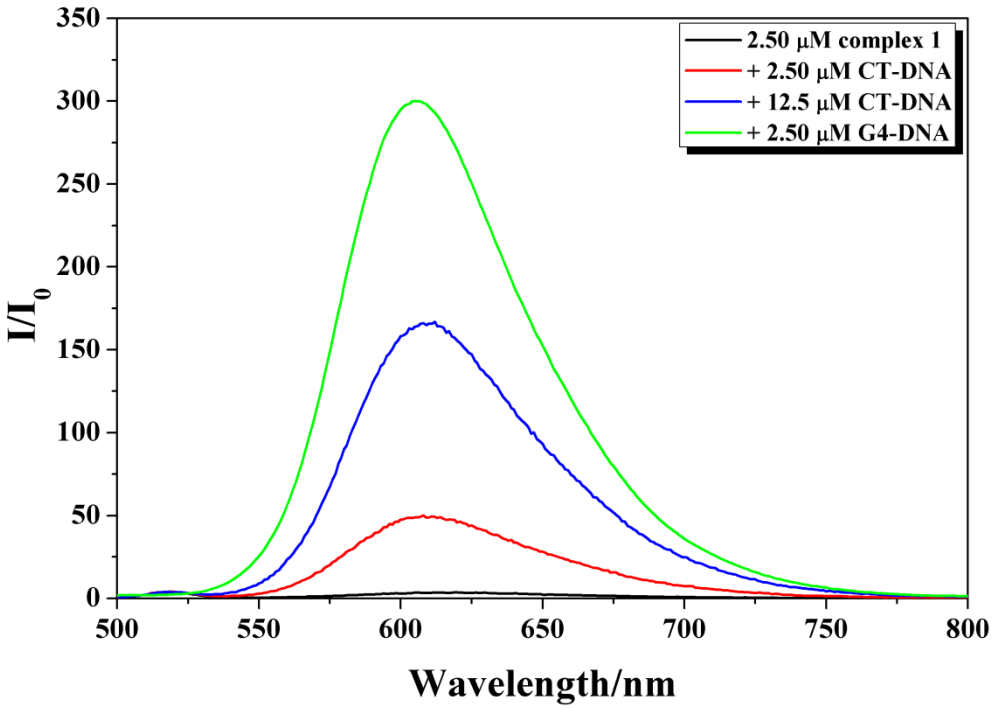

Fig. S4 Fluorescence spectrum of complex **1** (2.5 μM) in the absence of DNAs (black line), in the presence of 2.5 μM of calf thymus DNA (red line), 12.5 μM of calf thymus DNA (blue line), and 2.5 μM of quadruplex DNA (green line).

This journal is © The Royal Society of Chemistry 2013

3. Competition dialysis assay

Competition dialysis was conducted according to the procedure of Ragazzon and Chaires.[3, 4] Stock solutions of both quadruplex (22AG, 100 μM) and duplex (Calf thymus DNA, 100 and 1000 μM) DNA were prepared as discussed above in the FID section. For the competition dialysis assay, 200 mL of the dialysate solution (10 mM Tris-HCl, 100 mM KCl, pH 7.0) containing 1 μM ligand ($[Ru(bpy)_2dppz\text{-}idzo]^{2+}$) was placed into a beaker. A volume of 1.0 mL (at 100 μM monomeric unit for 22AG, 1000 μM for CT-DNA) of each of the DNA samples pipetted into a separate 1.0 mL Spectro/Por DispoDialyzer unit (MWCO: 3,500). All the dialysis units were then placed in the beaker containing the dialysate solution. The beaker was covered with Parafilm, and its contents were allowed to equilibrate with continuous stirring for 60 h at room temperature. At the end of the equilibration period, DNA samples were carefully removed to microfuge tubes, and were taken to a final concentration of 1% (w/v) Tween 80 by the addition of appropriate volumes of a 10% (w/v) stock solution. Concentrations of ligand bound to the DNA were calculated using extinction coefficient for the ligand determined in the same buffer: λ = 400 nm, ε = 32,000 $M^{-1}cm^{-1}$. The results were plotted as a bar graph using Origin software.

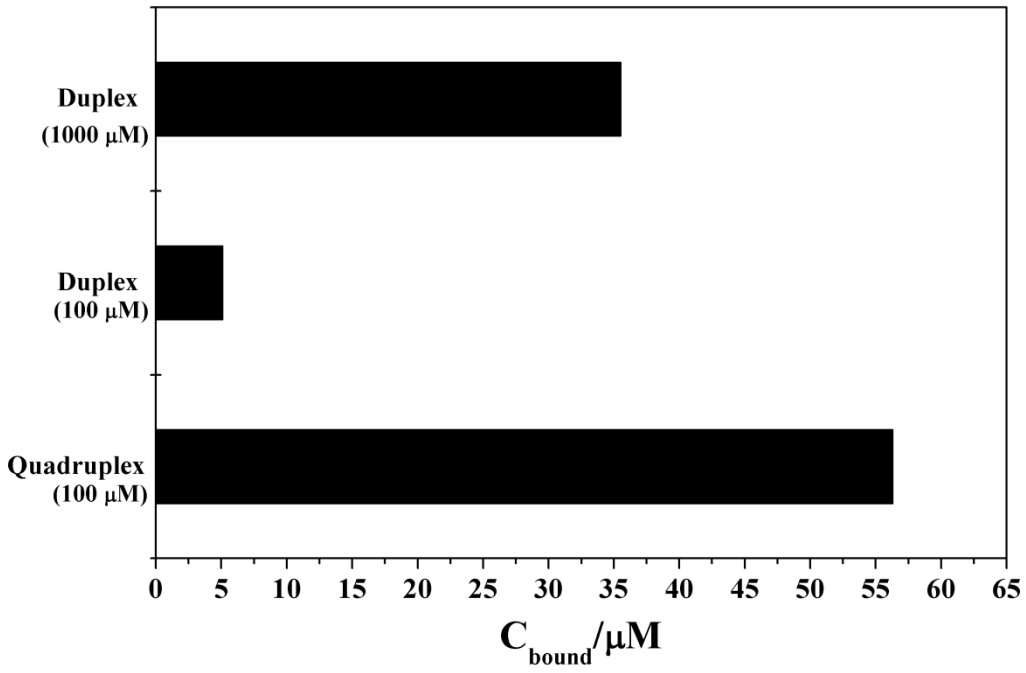

Fig. S5 Results obtained by the competition dialysis method in Tris-HCl buffer (10 mM Tris-HCl, 100 mM KCl, pH 7.0) containing 1 μM of complex **1**. The amount of complex **1** bound to each DNA structure is shown as a bar graph.

5. Computational calculations

This journal is © The Royal Society of Chemistry 2013

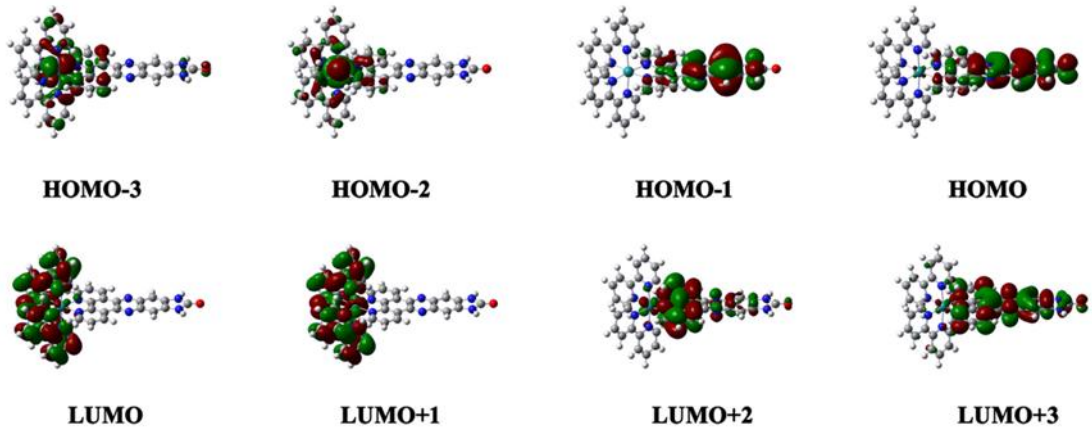

Fig. S6 Contour plots of some selected frontier molecular orbitals of complex $[Ru(bpy)_2dppz\text{-}idzo]^{2+}$ without consideration of water solvent.

Table S1. Selected calculated bond dihedral angels (deg) of $[Ru(bpy)_2dppz\text{-}idzo]^{2+}$ using the DFT-B3LYP at the Lanl2dz/6-31G* Level.

complex	dihedral angle (deg)			
	C_{67}-C_{70}-C_{69}-N_{76}	C_{64}-C_{69}-C_{70}-N_{72}	C_{70}-C_{69}-N_{76}-C_{71}	C_{70}-N_{72}-C_{71}-O_{75}
$[Ru(bpy)_2dppz\text{-}idzo]^{2+}$	179.9852	179.9828	0.00093	179.99535

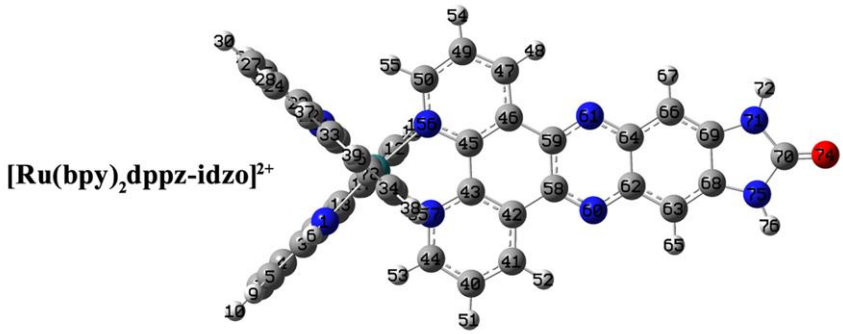

Fig. S7 Numbered structure of $[Ru(bpy)_2dppz\text{-}idzo]^{2+}$.

This journal is © The Royal Society of Chemistry 2013

Table S2. TD-DFT calculated energies, oscillator strengths, transition contributions, and coefficient of the six lowest-energy excited singlet states of $[Ru(bpy)_2dppz\text{-}idzo]^{2+}$ without consideration of water solvent. The nonemissive transitions are marked in red.

excited state	λ abs/nm(eV)	oscillator strength	transition contribution	coefficient
ES1 (BS)	482.57(2.57)	0.0127	HOMO→LUMO	0.69391(96.30%)
ES2 (BS)	470.87(2.63)	0.0013	HOMO-3→LUMO+1	-0.13570(3.68%)
			HOMO→LUMO+1	0.69137(95.60%)
ES3 (BS)	459.85(2.70)	0.0000	HOMO-2→LUMO+1	0.69689(97.13%)
ES4 (MS)	459.346(2.70)	0.0006	HOMO-2→LUMO	0.69293(96.03%)
			HOMO-2→LUMO+2	0.10935(2.39%)
ES5 (MS)	454.81 (2.73)	0.0014	HOMO-1→LUMO	0.64537(83.30%)
			HOMO-1→LUMO+2	-0.26794(14.36%)
ES6 (DS)	454.35(2.73)	0.0938	HOMO→LUMO+2	0.67842(92.05%)
			HOMO→LUMO+3	0.12651(3.20%)

References

1. R. B. Nair, E. S. Teng, S. L. Kirkland and C. J. Murphy, *Inorg Chem*, 1998, **37**, 139-141.
2. S. Shi, X. Geng, J. Zhao, T. Yao, C. Wang, D. Yang, L. Zheng and L. Ji, *Biochimie*, 2010, **92**, 370-377.
3. J. Ren and J. B. Chaires, *Biochemistry*, 1999, **38**, 16067-16075.
4. P. Ragazzon and J. B. Chaires, *Methods*, 2007, **43**, 313-323.