Electronic supplementary information

Molecular "light switch" for G-quadruplexes DNA: Cycling the Switch On and Off

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Synthesis

1,10-phenanthroline-5,6-dione,[1] 5,6-Dinitrobenzimidazole,[2] 5,6-diaminobenzimidazole[3] and cis-$Ru(bpy)_2Cl_2.2H_2O$ (bpy = 2,2'-bipyridine)[4] were synthesized according to the literature methods. The other chemicals were obtained from commercial sources and used without further purification. $[Ru(bpy)_2(dppzi)]^{2+}$ was synthesized according to a route shown in Scheme 1, and the synthetic details are given below.

Dipyrido[3,2-a:2',3'-c]phenazine-10,11- imidazole (dppzi)

A mixture of 0.42 g (2.0 mmol) of 1,10-phenanthroline-5,6-dione and 0.30 g (2.0 mmol) of 5,6-diaminobenzimidazole in 20 mL of methanol was refluxed for 2 h. Upon cooling, the yellow precipitate was collected by filtration and further recrystallized from methanol. Yield 78%. Anal. Calcd for $C_{19}H_{10}N_6$: C, 70.80; H, 3.13; N, 26.07. Found: C, 70.76; H, 3.15; N, 26.05. MS: m/z 322.1 $(M)^+$.

$[Ru(bpy)_2dppzi](PF_6)_2$

Cis-$[Ru(bpy)_2Cl_2].2H_2O$ (160 mg, 0.30 mmol) and dppzi (94 mg, 0.30 mmol) were added to 20 ml ethylene glycol–water (9 : 1, v/v). The mixture was refluxed for 6 h under an argon atmosphere. The cooled reaction mixture was diluted with water (50 ml) and filtered to remove solid impurities. The filtrate was added ammonium hexafluorophosphate. The precipitated complex was dried, dissolved in a small amount of acetonitrile, and purified by chromatography over alumina, using MeCN–toluene (3 : 1, v/v) as eluent and further recrystallized from acetone/diethyl ether (1:5, v/v). Yield: 250 mg, 81%. ^{1}H NMR [$(CD3)_2SO$]: δ 13.27(1H, s), 9.67(2H, d), 8.90(5H, t), 8.67 (2H, t), 8.23(4H, t), 8.14(2H, t), 8.02(2H, t), 7.82(4H, dd), 7.61(2H, t), 7.39(2H, t). ^{13}C NMR [$(CD3)_2SO$]: δ 157.3, 156.9, 153.3, 152.3, 151.9, 150.5, 138.5, 138.4, 133.4, 131.0, 128.4, 128.2, 128.0, 125.0, 124.8. Calc. for $C_{39}H_{26}F_{12}N_{10}P_2Ru$: C, 45.67; H, 2.56; N, 13.66. Found: C, 45.64; H, 2.57; N, 13.68. ESI-MS: *m*/*z* 367.4 ($M-2PF_6/2$).

Materials and methods:

DNA oligomers 5′-AGGGTTAGGGTTAGGGTTAGGG-3′ (22AG) and 5′-CCCTAACCCTAACCCTAACCCT-3′ (I-motif) were purchased from Sangon (Shanghai, China) and used without further purification. CT-DNA was purchased from the Sino-American Biotechnology Company. Concentrations of these oligomers were determined by measuring the absorbance at 260 nm after melting. Single-strand extinction coefficients were calculated from mononucleotide data using a nearest-neighbor approximation.[5] The formations of intramolecular G-quadruplexes were carried out as follows: the oligonucleotide samples, dissolved in different buffers, were heated to 90 °C for 5 min, gently cooled to room temperature, and then incubated at 4 °C overnight. Buffer A: 100 mM NaCl, 10

mM NaH_2PO_4/Na_2HPO_4, 1 mM Na_2EDTA, pH 7.0; Buffer B: 100 mM KCl, 10 mM KH_2PO_4/K_2HPO_4, 1 mM K_2EDTA, pH 7.0.

Elemental analyses (C, H and N) were carried out with a Perkin–Elmer 240C elemental analyzer. An LCQ electrospray mass spectrometer (ESMS, Finnigan) was employed for the investigation of charged metal complex species in CH_3CN solvent.

Absorption spectra titrations: Absorption spectra titrations were carried out at room temperature to determine the binding affinity between DNA and complex. Initially, 3000 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 200-600 nm. During the titration, aliquot (1-10 μL) of buffered DNA solution was added to each cuvette to eliminate the absorbance of DNA itself, and the solutions were mixed by repeated inversion. After the solutions were mixed for ~5 minutes, the absorption spectra were recorded. The titration processes were repeated until there was no change in the spectra for four titrations at least, indicating binding saturation had been achieved. The changes in the metal complex concentration due to dilution at the end of each titration were negligible.

In order to compare quantitatively the binding strength of $[Ru(bpy)_2(dppzi)]^{2+}$ to each G-quadruplex DNA, the intrinsic binding constants K_b with each DNA at 25 °C were obtained using the following eq. (1), [6-8]

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

$$b = 1 + KC_t + K[\mathrm{DNA}]/2s \quad (1b)$$

where [DNA] is the concentration of DNA in base pair, 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. K is the equilibrium binding constant in M^{-1}, C_t is the total metal complex concentration, and s is the binding size.

Thermal DNA denaturation experiments: Thermal DNA denaturation experiments were carried out with a PerkinElmer Lambda 850 spectrophotometer equipped with a Peltier temperature-control programmer (± 0.1) °C. Melting curves were collected by UV absorbance as a function of temperature. The temperature of the solution was increased from 40 to 90 °C at a rate of 1°C/min, and the absorbance at 295 nm was continuously monitored for solutions of DNA (5.0 μM) in the absence and presence of the Ru(II) complex (5.0 μM). The data were presented as $(A - A_0)/(A_f - A_0)$ versus temperature, where A_f, A_0, and A were the final, the initial, and the observed absorbance at 295, respectively.

Emission spectra titrations: Emission spectra were measured on a Shimadzu RF-5000 spectrofluorophotometer. The excitation wavelength was 440 nm, and the emission spectrum was collected from 500 to 750 nm. Excitation and emission slits were set at 10 and 10 nm, respectively. Luminescence titrations process was similar to CD titration experiment. Luminescence titrations: A 3000 μL of 5 μM $[Ru(bpy)_2(dppzi)]^{2+}$ in a 1.0 cm path length quartz cuvette was loaded into the fluorimeter sample block, maintained at 25 °C. After 5 minutes to allow the cell to equilibrate, the first spectrum was recorded, and then 1-10 μL of DNA solution was then added to the sample cell, followed by thorough mixing. After 5 minutes, the spectrum was taken again. 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 binding constant K_b can be determined by the following equation: [9,10]

$$\text{Log } (F-F_0)/F = \log K_b + n \log [DNA] \qquad (2)$$

where F_0 and F represent the steady-state fluorescence intensities in the absence and presence of DNA. K_b is the binding constant and n is the number of binding sites per DNA, which can be determined by the intercept and slope of double logarithm regression curve of log $(F-F_0)/F$ versus log[DNA] based on the equation. To evaluate the thermodynamic parameters, fluorimetric titration of $[Ru(bpy)_2(dppzi)]^{2+}$ with G-quadruplex DNA was performed at 15, 30, 37 and 45 °C, respectively.[11,12] The standard enthalpy, standard entropy, and standard free-energy change of the binding of $[Ru(bpy)_2(dppzi)]^{2+}$ to G-quadruplex DNA were determined by van't Hoff's Equations (3), (4), and (5), where R is the gas constant (R = 1.987 cal mol^{-1} deg^{-1}) and T is the temperature in Kelvin; K_1 and K_2 are the DNA-binding constants of the metal complex at temperatures T_1 and T_2, respectively, and ΔH^0, ΔG^0, and ΔS^0 the standard enthalpy, standard free-energy, and standard entropy change of the metal-complex-binding to DNA, respectively.

$$\ln(\frac{K_1}{K_2}) = \frac{\Delta H^0}{R}(\frac{T_1 - T_2}{T_1 T_2}) \qquad (3)$$

$$\Delta G^0 = -RT \ln K \qquad (4)$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad (5)$$

Quenching studies: Fluorescence quenching studies were carried out using the anionic quencher potassium ferrocyanide ($K_4Fe[CN)_6]$), monitoring the fluorescence intensity changes at 625 nm as a function of the quencher concentration. At least four measurements were taken and averaged. The data were plotted as I^o/I versus quencher concentration [Q] according to the Stern–Volmer equation, as described earlier.[13]

Molecular docking studies: Both the antiparallel basket quadruplex (PDB ID 143D) and the mixed parallel/antiparallel structure (PDB ID 2HY9) were used as an initial model to study the interaction between $[Ru(bpy)_2(dppzi)]^{2+}$ and 22-mer telomeric G-quadruplex DNA. $[Ru(bpy)_2(dppzi)]^{2+}$ was optimized using DFT with a LanL2MB basis set.[14] The optimized structure of the ruthenium complex was used to do the docking. When prepare for Docking DNA and ligand ($[Ru(bpy)_2(dppzi)]^{2+}$) PDB file (using Auto Dock Tools in AutoDock), necessary modifications were carried out including: (1) Add all hydrogens or just non-polar hydrogens; (2) Assign partial atomic charges to the ligand and the macromolecule (Gasteiger or Kollman United Atom charges). (3) Merge non-polar hydrogens and Set up rotatable bonds in the ligand; (4) output PDBQT files from traditional PDB files are also created for the side chain coordinates. Ligand docking was carried out with the AutoDock 4.2 Lamarckian Genetic Algorithm (LGA).[15-17] For antiparallel basket G-quadruplex structure, to create a pseudo-intercalation ligand binding site between the diagonal loop and the G-quartet segment of the structure (at the 5' AG step) in the human intramolecular G-quadruplex NMR structure (PDB code 143D), the following steps have been employed: (1) Two phosphate backbones are broken at the 5' AG step; (2) The two halves of the structure are separated so that the separation of the A:A base pair and the G-quartet is increased from 3.4 to 6.8 Å; (3) The sugar-phosphate backbones are reconnected.[18] As for the mixed hybridtype G-quadruplex structure, two adenines from each end of the mixed hybrid-type structure (PDB code 2HY9) were removed and similar operations to increase the separation between loop base pairs and the G-quartet were performed.[19] In the autodocking, DNA was enclosed in the grid defined by Auto Grid having 0.375 Å spacing and parameters (supplied with the program package) were used for dispersion/repulsion, hydrogen bonding, electrostatics, and desolvation, respectively. Auto Grid performed a precalculated atomic affinity grid maps for each atom type in the ligand plus an electrostatics map and a separate desolvation map present in the substrate molecule. Then, during the AutoDock calculation, the energetics of a particular ligand configuration is evaluated using the values from the grids. The output from AutoDock was rendered with Accelrys Discovery Studio 3.0 Client.[20]

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Table s1

Absorption spectra (λ_{max}/nm) of complex $[Ru(bpy)_2(dppzi)]^{2+}$ in 100 mM K^+ and Na^+ buffer solutions.

G-4	λ_{max}/free	λ_{max}/bound	$\Delta\lambda$/nm	*H* (%)	$K_b/10^5 M^{-1}$	*s*
mixed	287	287	0	26.07		
parallel/antiparallel quadruplex	386	389	3	40.18	9.5 ± 2.3	1.3 ± 0.1
	431	435	4	18.59		
antiparallel basket quadruplex	287	287	0	21.40		
	386	389	3	35.00	5.1 ± 2.4	1.0 ± 0.2
	430	434	4	15.85		

Table s2. Thermodynamic parameters and binding constants for the binding of $[Ru(bpy)_2(dppzi)]^{2+}$ to G-quadruplexes DNA.

G-4	T (K)	log K (M^{-1})	ΔG/ (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (cal deg^{-1} mol^{-1})
antiparallel basket quadruplex	288	6.9	-9.1		
	303	5.2	-7.2	-31.8	-78.8
	310	4.1	-5.8		
	318	2.2	-3.2		
mixed parallel/antiparallel structure	288	7.8	-10.2		
	303	5.6	-7.7	-36.0	-89.5
	310	4.3	-6.1		
	318	2.5	-3.6		

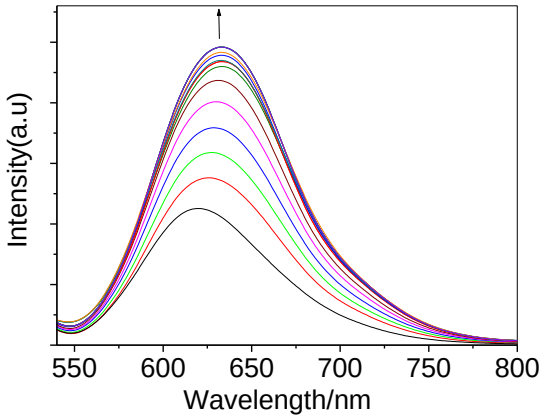

Fig. s1. Emission spectra of $[Ru(bpy)_2(dppzi)]^{2+}$ in the presence of increasing amounts of CT DNA, [Ru] = 5 μM, [DNA] = 0–50 μM in 100 mM K^+ buffer. Arrow shows the emission intensity changes upon increasing DNA concentrations.

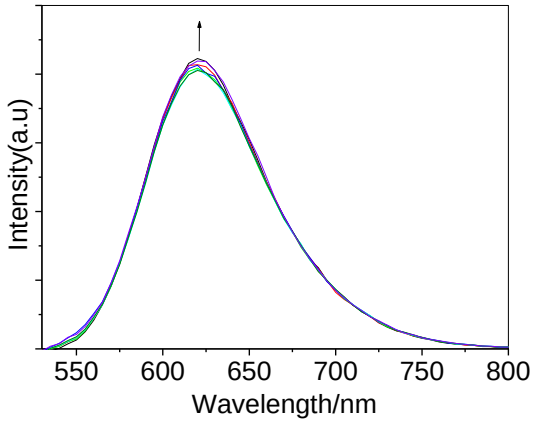

Fig. s2. Emission spectra of $[Ru(bpy)_2(dppzi)]^{2+}$ in the presence of increasing amounts of I-motif DNA, [Ru] = 5 μM, [DNA] = 0–50 μM in 100 mM K^+ buffer. Arrow shows the emission intensity changes upon increasing DNA concentrations.