Research paper

Interaction of [Ru(bpy)2(dppz)]2+ with human telomeric DNA: Preferential binding to G-quadruplexes over i-motif

Shuo Shi a,*, Xiaoting Geng a, Juan Zhao a, Tianming Yao a, Chaoran Wang a, Danjing Yang a, Lengfeng Zheng a, Liangnian Ji a,b

Abstract

Inspired by the enormous importance attributed to the structure and function of human telomeric DNA, we focus our attention on the interaction of [Ru(bpy)2(dppz)]2+ with the guanine-rich single-strand oligomer 5′-AGGTTAGGTTAGGTTAGG-3′ (22AG) and the complementary cytosine-rich strand (22CT). In Na+ buffer, 22AG may adopt an antiparallel basket quadruplex, whereas, it favours a mixed parallel/antiparallel structure in K+ buffer. 22CT may self-associate at acidic pH into an i-motif. In this paper, the interaction between [Ru(bpy)2(dppz)]2+ and each unusual DNA was evaluated. It was interesting that [Ru(bpy)2(dppz)]2+ could promote the human telomeric repeat 22AG to fold into intramolecular antiparallel G-quadruplex without any other cations. What’s more, [Ru(bpy)2(dppz)]2+ was found to have a strong preference for binding to G-quadruplexes that were induced through either Na+ or K+, while weak binding to i-motif was observed. The results also indicated that [Ru(bpy)2(dppz)]2+ could serve as a prominent molecular “light switch” for both G-quadruplexes, revealing a potential application of the title complex in luminescent signaling of G-quadruplex DNA.

© 2010 Elsevier Masson SAS. All rights reserved.

1. Introduction

Telomeres are specific nucleoprotein structures, which form a ‘cap’ at the end of eukaryotic chromosomes. When this distal ‘cap’ is disrupted by adding mutant repeats or shortening the existing telomere beyond a critical length, the chromosome becomes uncapped. Uncapping of the telomere ends leads to telomeric dysfunction characterized by end-to-end fusion, inappropriate recombination, anaphase bridges, and G-overhang degradation that may lead to either apoptosis or senescence [1–4]. So telomeres are essential for chromosomal stability and genome integrity, which provide sites for recombination events and transcriptional silencing, and appear to play a critical role in cellular aging and cancer [5,6]. The telomeric DNA has a unique mode of replication based on a special reverse transcriptase enzyme called telomerase.

This enzyme maintains telomeric DNA integrity and prevents critical telomere shortening with successive cell division by catalysing the synthesis of telomeric DNA so that cells cannot reach crisis points of senescence and apoptosis. Telomerase is up-regulated in 80–85% of tumor cells in comparison to normal somatic cells and is a major factor in cancer cell immortalisation. This unique activity of telomerase makes the enzyme an ideal probe for tumor diagnosis and a target for cancer chemotherapy. Therefore, currently there is considerable interest in finding molecules that can inhibit telomerase and potentially act as anti-cancer drugs. A number of distinct methods to inhibit the enzyme and its unique mechanism of telomeric DNA catalysis have been suggested [7–11]. Significantly, formation of a quadruplex DNA structure is a promising approach, which is incompatible with telomerase attachment to the 3′ end, suggesting that quadruplex-binding and quadruplex-promoting small molecules can in principle effectively inhibit both the catalytic and capping functions of telomerase. Furthermore, stabilization of G-quadruplex structures by small molecules directly causes telomeres uncapping and prevents elongation of telomeres by disrupting the interaction between the enzyme and its substrate [1–4,11–14].

Human telomeres consist of tandem repeats of the double-stranded DNA sequence (5′-TTAGGG); (5′-CCCTAA). The G-rich strand can form polymorphic quadruplexes containing G-quartets...
stabilized by Hoogsteen hydrogen bondings and the complementary C-rich strand may self-associate at acidic or even neutral pH into an intercalated structure called the i-motif based on intercalated C–C+ base pairs (Fig. 1) [15–19]. Compared to G-quadruplexes, i-motif has a much shorter history. It was first observed by NMR in the tetramer of d(TCCCCC) in 1993 [20]. The biological importance of the intramolecular i-motif is evidenced by its involvement in human telomeric and centromeric DNA structures and RNA intercalated structures, and by the discovery of several proteins that bind specifically to C-rich telomeric DNA fragments capable of forming i-motifs. Along with human telomeric G-quadruplex DNA, the i-motif has also been an attractive drug target for cancer chemotherapy and for modulation of gene transcription [16]. Because of the biological implications, such nonduplex structures are emerging as a new class of therapeutic targets for cancers and other diseases. Owing to the increasing number of G-quadruplexes and i-motif being identified in the human genome, an emerging goal is to elucidate G-quadruplex and i-motif recognition by small molecules.

G-quadruplex and i-motif DNA are highly dynamic and polymorphic DNA structures, making it a particular challenge to target in rational drug design. The structures and the stability of the G-quadruplex depend on monocations, for example, the NMR structure of AG3(T2AG3)3 in the presence of Na+ was an antiparallel basket quadruplex [21], but the X-ray structure for the same sequence in the presence of K+ revealed a parallel propeller quadruplex [22]. A recent paper indicated that it favored a mixed parallel/antiparallel structure in the presence of K+ solution based on circular dichroism [23,24] (Fig. 1A). However, the structures and the stability of the i-motif are sensitive to pH [16–19]. Consistent with the hemiprotonated base pairs, the stability of the i-motif is at a maximum at a pH value close to the pKₐ value of cytosine. At a neutral pH value, the i-motif is much less stable although it can still be observed in intramolecular systems [25]. Owing mainly to its specific self-recognition and susceptibility to pH variation, this cytosine-rich tetraplex has been utilized creatively as building components for fabricating various molecular devices and machines [26,27]. Nevertheless, since targeting via specific ligands

![Fig. 1](image-url)
is emerging as a novel anti-cancer strategy [11–19], considerable scientific efforts for designing and synthesizing novel and efficient G-quadruplex and i-motif ligands have been taken in the past few years. In general, an effective G-quadruplex binder possesses a large electron deficient π-aromatic surface, positively charged substituents that can interact with the grooves of the quadruplex, as well as a positively charged center which can reside near the center of the guanine quartet. Many elegant studies on organic G-quadruplex binders have appeared [11–14]. Recently, a number of metal ion complexes were evaluated for G-quadruplex binding. The metal can play a major structural role in organizing the ligand (s) into an optimal structure for quadruplex DNA interaction. Also, the electropositive metal can in principle be positioned at the center of the guanine quartet, increasing electrostatic stabilization by substituting the cationic charge of the potassium or sodium that would normally occupy this site. Recent results reporting on the high performances of complexes to target G-quadruplex gave much impetus to this approach [15,28].

The complexation of a quadruplex DNA will result in an increase of the base stacking and stabilization of the DNA [39]. Only two molecules that can stabilize both G-quadruplex and i-motif DNA have been identified [40,41]. Recently, carboxyl-modified single-walled carbon nanotubes selectively induced human telomeric i-motif formation [42]. To the best of our knowledge, a complex that can selectively target G-quadruplex DNA over i-motif DNA has not been reported.

RuII complexes have prominent DNA-binding properties. In particular, [Ru(bpy)2(dpdz)]2+ (dpdz = dipiryridino[2,3-a;2',3'-c]phenazine) (Fig. 1B) known as DNA “light switch” has attracted particular attention. It can intercalate between the duplex DNA base pairs and stabilize the DNA [42–44]. It is worth pointing out that the use of [Ru(bpy)2(dpdz)]2+ for G-quadruplex and i-motif DNA recognition is still under-developed in contrast to its considerable use as duplex DNA binders. Herein, human telomeric fragments of 5′−AGGGTTAGGGTAGGGG−3′ and 5′−CCCTAACCTAGGGTATGG−3′ (denoted 22AG and 22CT, respectively) are chosen for this investigation, whose crystal and solution structures have been achieved. The changes in the metal complex concentration [Ru(bpy)2(dpdz)]2+ were measured by titration processes were repeated until there was no change in the absorbance at 260 nm after melting. Single-strand extinction coefficients were calculated using a Scatchard plot [48] of f r/C r versus f r, where f r is the free complex concentration.

2.3. Emission spectra

Emission spectra were measured on a Shimadzu RF-5000 spectrofluorometer. The excitation wavelength was 460 nm, and the emission spectrum was collected from 500 to 800 nm. Excitation and emission slits were set at 10 and 10 nm, respectively. Luminescence titration process was similar to CD titration experiment. Luminescence titrations: A 3000 μL of [Ru(bpy)2(dpdz)]2+ in a 1.0 cm path length quartz cuvette was loaded into the fluorimeter sample block. After 5 min, 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.

2.4. 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 (6 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 [Ru(bpy)2(dpdz)]2+ solution was added to the cuvette, and the solutions were mixed by repeated inversion. After the solutions were mixed for ~5 min, the CD spectra were recorded. The titration processes were repeated until there was almost no change, indicating binding saturation had been achieved. For each sample, at least four spectrum scans were accumulated over the wavelength range of 200−350 nm at the temperature 25°C in a 1.0 cm path length cell at a scanning rate of 50 nm/min. The instrument was flushed continuously with pure evaporated nitrogen throughout the experiment. The scan of the buffer alone was subtracted from the average scan for each sample.

2.5. 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. Absorbance changes at either 295 nm (G-quadruplex) or 260 nm (i-motif) vs. temperature were collected [16] at a heating rate of 1 °C/min. The data were presented as (A – A0)/(A1 – A0) versus temperature, where A0, A1, and A were the final, the initial, and the observed absorbance at 295 or 260 nm, respectively.

2.6. Competitive dialysis

For each competition dialysis assay, 400 mL of dialysate solution containing 1 µM [Ru(bpy)2(dppz)]2+ was placed into a beaker. A volume of 0.5 mL (at 10 µM monomeric unit) of each of the DNA samples was pipetted into a separate 0.5 mL dialysis cassette (Pierce). The entire dialysis cassettes were then placed in the beaker containing the dialysate solution. The contents were allowed to equilibrate with continuous stirring for 24 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) sodium dodecyl sulfate (SDS). The total concentration of [Ru(bpy)2(dppz)]2+ within each dialysis cassette was then determined spectrophotometrically using a wavelength of 445 nm. The free complex concentration (Cf) was determined spectrophotometrically using an aliquot of the dialysate solution. The amount of bound [Ru(bpy)2(dppz)]2+ was determined by the difference between the total complex concentration and the free complex concentration (Cf = C0 – Cf).

CD titrations, luminescence titrations, absorption spectra titrations, thermal denaturation profiles, as well as competitive dialysis for every sample were repeated at least three times.

3. Results and discussion

Circular dichroism (CD) spectroscopy was used to characterize the solution conformations of 22AG and 22CT. Without any metal cations, the CD spectra of 22AG at room temperature exhibited a negative band centered at 235 nm, a major positive band at 257 nm, which probably corresponded to the signal of the random coil. 22AG (characterized by a positive peak at 257 nm) [49]. Upon addition of [Ru(bpy)2(dppz)]2+ to 22AG aqueous solution (Fig. 2A), a significant change in the CD spectrum was observed. With the increase of [Ru(bpy)2(dppz)]2+, the maximum at 257 nm was gradually suppressed and shifted to 245 nm, while a major negative band at about 263 and a major positive band at 295 nm started to appear. These significant changes in the CD spectrum of 22AG after the addition of [Ru(bpy)2(dppz)]2+ were consistent with this complex binding to the DNA and thus causing substantial change(s) in the conformation of the DNA. Upon addition of 20 µM [Ru(bpy)2(dppz)]2+ to 22AG, the CD spectrum of this new DNA conformation was similar to the CD spectra of antiparallel G-quadruplexes described in previous studies, where the major positive band was usually observed around 295 nm with a negative band at 265 nm and a smaller positive band at 246 nm [49]. It indicated that [Ru(bpy)2(dppz)]2+ induced the formation of the human telomeric intramolecular G-quadruplex structure without any other metal ions. However, upon addition of [Ru(bpy)2(dppz)]2+ to 22CT, no obvious spectral changes were observed in the same condition (Fig. 2B), which implied that [Ru(bpy)2(dppz)]2+ could not induce the formation of i-motif at pH 7.0.

The structures and the stability of the G-quadruplexes depend on monocations, such as Na+ and K+, but pH-independent; whereas the structures and the stability of the i-motif are sensitive to pH, but monocations-independent [18]. As shown in Fig. s1 [13], C-rich strand was strongly pH-dependent, at pH 5.5, the CD spectra showed a large positive band with a peak at 286 nm and a negative band centered at 254 nm. This type of spectrum has been attributed to the formation of C–C+ base pairs, characteristic of the i-motif structure [16–19,50].

Therefore, the structures of G-quadruplex and i-motif were investigated in the buffer (A) and buffer (B) at pH 5.5. Upon addition of [Ru(bpy)2(dppz)]2+ to 22AG in Na+ or K+ buffered solution, the CD spectrum exhibited a maxima-minima pattern similar, but not identical to the spectrum in Na+ or K+ without addition of [Ru(bpy)2(dppz)]2+ (Fig. 2C, D), which implied that the conformation of G-quadruplex was stabilized by Na+ or K+, and [Ru(bpy)2(dppz)]2+ could not change the conformation of G-quadruplex at high ionic strength. In contrast, as the [Ru(bpy)2(dppz)]2+ was gradually added into 22CT at pH 5.5, a moderate increase in the magnitude of the ellipticity at 286 nm was observed (Fig. 2E), which implied that [Ru(bpy)2(dppz)]2+ favored the conformation of i-motif.

To further clarify the nature of the interaction between the complex and DNA, luminescence measurements were carried out. Fig. 3A showed the relative emission intensities I0/I0 (where I0 was the emission measured in the absence of oligonucleotide) for [Ru(bpy)2(dppz)]2+ in the presence of 22AG or 22CT. As expected, [Ru(bpy)2(dppz)]2+ showed negligible luminescence in buffered solutions. Addition of 22AG to complex (either in K+ or Na+ buffered solutions) resulted in a “light switch” effect (Fig. 3A). In this case an emission enhancement in K+ buffered solution induced through 22AG was about 4 times larger than that occurred in Na+ buffered solution, which implied that [Ru(bpy)2(dppz)]2+ bound mixed parallel/antiparallel structure quadruplex more avidly than anti-parallel basket quadruplex. But the increase of luminescence was pH-independent, for example, emission enhancement induced through 22AG in K+ buffered solution at pH 7.0 was almost the same as that at pH 5.5 (Fig. S2).

Compared to both G-quadruplexes DNA, i-motif DNA can slightly increase the fluorescence of the complex. Based on the luminescence emission enhancement, the intrinsic binding constant was obtained according to the Scatchard equation. The values of binding constant were about 105, 106 and 104 for mixed parallel/antiparallel structure quadruplex, antiparallel basket quadruplex and i-motif, respectively. The binding constants indicated that the interaction between [Ru(bpy)2(dppz)]2+ and DNA was strong and [Ru(bpy)2(dppz)]2+ interacted preferentially with both G-quadruplexes structures over i-motif DNA. It was clear that the interaction between the complex and DNA depended on ionic strength. Fig. 3B illustrated that the DNA-binding constant of [Ru(bpy)2(dppz)]2+ decreased with the concentration of added ions. This was due to the stoichiometric amount of counterion released that followed the binding of charged complex, suggesting that electrostatic interaction was involved in the DNA-binding event [51].

Absorption spectra titrations were performed to determine the binding affinity of complex to 22AG and 22CT, too. DNA sample was added in aliquots sequentially to complex solutions, with absorbance spectra recorded after each addition. The changes in the spectral profiles during titration were shown in Fig. 4. As the concentration of DNA increased, the absorbance in the ligand absorption region, as well as the MLCT band decreased. Addition of 22AG to a solution of [Ru(bpy)2(dppz)]2+ in a K+ buffer led to a 11 nm red shift and 19.1% hypochromism of the band at 444 nm; however addition of 22CT to the same buffered solution led no red shift and 10.5% hypochromism of the band at 444 nm. Isosbestic points were located at 301 and 386 nm. Hypochromism and red shift indicated strong interactions between the DNA bases and the complexes. The similar case could be seen in Na+ buffered solution, too (Fig. S3). The exact data were shown in Table 1.

In order to compare quantitatively the binding strength of [Ru(bpy)2(dppz)]2+ to each DNA, the intrinsic binding constants Ki
with each DNA at 25 °C were obtained using the following Eq. (2) [52–54],

\[
\frac{(\epsilon_a - \epsilon_t)}{(\epsilon_b - \epsilon_t)} = \left( b - (b^2 - 2K^2C_t[DNA]/s)^{1/2} \right) / 2KC_t \quad (2a)
\]

\[
b = 1 + K\epsilon_i + K[DNA]/2s \quad (2b)
\]

where [DNA] is the concentration of DNA in base pair, \(\epsilon_a\), \(\epsilon_b\), and \(\epsilon_t\) 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. The values of binding constant were comparable to the values obtained from luminescence titration with McGhee-von Hippel method. Both sets of binding constants indicated that the complex \([\text{Ru(bpy)}_2(\text{dppz})]^2^+\) bound more tightly to both G-quadruplexes than i-motif.

To determine the selectivity of the interaction for the G-quadruplex relative i-motif DNA, UV melting was used to study how the complex affected the stability of the human telomeric DNA. The stability of i-motif was the same in Na\(^+\) and K\(^+\): the melting temperatures were identical in 100 mM NaCl and 100 mM KCl, but the melting of the C-rich strand was strongly pH-dependent. However, the melting temperature of the G-quadruplex was pH-independent in the pH 4.5–7.5 range, but counterion-dependent, with a \(T_m\) of 63 °C in 100 mM KCl and 58 °C in 100 mM NaCl [18]. So the stability of G-quadruplex and i-motif was investigated in the buffer (A) and buffer (B) at pH 5.5. Consistent with previous studies [16], 295 nm and 260 nm were chosen to study the influence of the complex on the stability of G-quadruplex DNA and i-motif DNA respectively.

The transition temperature of the G-quadruplex increased from 55.2 to 60.5 °C in Na\(^+\) buffer and increased from 65.1 to 69.8 °C in K\(^+\) buffer, in which an increase in the melting temperature of the
A drawback that the lack of specificity for quadruplex indicated a stabilizing effect (Fig. 5A), however [Ru(bpy)2(dppz)]2+ had little influence on the thermal stability of i-motif either in Na+ buffer or in K+ buffer (Fig. 5B). Such a result also confirmed the above proposal that [Ru(bpy)2(dppz)]2+ interacted preferentially with G-quadruplex.

To evaluate the selectivity of [Ru(bpy)2(dppz)]2+ for different DNA structures, we performed a competitive dialysis experiment using different types of DNA against a common complex solution. Among the DNA used in the present study, 22AG could form the G-quadruplex structure, 22CT could form the i-motif structure, and calf thymus DNA was a native duplex DNA structure. More products accumulated in the dialysis cassette containing the structural form with the highest complex binding affinity [55,56]. Competition dialysis results for [Ru(bpy)2(dppz)]2+ were shown as a bar graph in which the amount of complex bound was shown for each DNA included in the assay. Though [Ru(bpy)2(dppz)]2+ showed prominent G-quadruplex binding affinity, a modest selectivity for quadruplex over duplex was observed in K+ buffered solution (Fig. 6). The similar case could be seen in Na+ buffered solution, too (Fig. 5B).

A similar complex [PtII(dppz-COOH)(N^C)]CF3SO3 [28] contained a dppz ligand with a pendant COOH functional group was found to have a strong preference for binding to G-quadruplexes, which might involve in H-bonding interaction with the guanine in the external tetrad of G-quadruplex DNA. What's more, dinuclear RuII complexes based tppz displayed preferences for quadruplex over duplex [38]. This indicated that modifying the complex could create some interesting differences in the DNA-binding properties, therefore, such structural information of the complexes was still important for a more comprehensive understanding of the biological implications of these structures and for designing new drugs.

Table 1

<table>
<thead>
<tr>
<th>Complex</th>
<th>DNA</th>
<th>λmax/free</th>
<th>λmax/bound</th>
<th>Δλ/μm</th>
<th>H (%)</th>
<th>Kd/10^7 M^-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ru(bpy)2(dppz)]2+</td>
<td>22AG (K+)</td>
<td>444</td>
<td>445</td>
<td>1</td>
<td>19.1</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>358</td>
<td>359</td>
<td>1</td>
<td>24.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>282</td>
<td>284</td>
<td>2</td>
<td>22.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22CT (K+)</td>
<td>444</td>
<td>444</td>
<td>0</td>
<td>10.5</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>358</td>
<td>358</td>
<td>0</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>282</td>
<td>283</td>
<td>1</td>
<td>15.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22AG (Na+)</td>
<td>444</td>
<td>445</td>
<td>1</td>
<td>17.9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>358</td>
<td>359</td>
<td>1</td>
<td>22.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>282</td>
<td>283</td>
<td>1</td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>22CT (Na+)</td>
<td>444</td>
<td>444</td>
<td>0</td>
<td>11.6</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>358</td>
<td>358</td>
<td>0</td>
<td>15.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>282</td>
<td>283</td>
<td>1</td>
<td>15.9</td>
<td></td>
</tr>
</tbody>
</table>
with enhanced activity and minimized undesired toxicity. On the other hand, dramatic preference for quadruplex over i-motif was observed. These equilibrium dialysis measurements nicely complemented the luminescence data to indicate that 

\[ [\text{Ru(bpy)}_2(dppz)]^{2+} \]

exhibited a significant preference for the G-quadruplexes over the i-motif.

### 4. Conclusions

Small molecules can potentially bind to a quadruplex or i-motif by externally stacking below the quartets, intercalating between the quartets, or nonspecifically binding to some random location on the DNA strand [57]. Given the structures of two G-quadruplexes and i-motif, we propose that 

\[ [\text{Ru(bpy)}_2(dppz)]^{2+} \]

may bind to these G-quadruplexes and is stacked on the ends of the G-quadruplexes like the similar complex [Pt^{IV}(dppz-COOH)(N^C)] CF_3SO_3 [28]; whereas complex may bind to i-motif DNA by nonspecific binding or by interaction with negatively charged DNA backbone because of weaker binding affinity. The results indicate that 

\[ [\text{Ru(bpy)}_2(dppz)]^{2+} \]

can serve as a prominent molecular “light switch” for both G-quadruplexes, and it preferentially binds to G-quadruplexes induced through either Na\(^+\) or K\(^+\) over an i-motif. The details of the binding modes are not clear yet and deserve further investigation.

### Acknowledgments

The authors thank the National Natural Science Foundation of China (20901060, 20871094), the Program for Young Excellent Talents in Tongji University and Students Innovation Training Program (STIP) for their support of this research.

### Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biochi.2010.01.003.

### References


---

**Fig. 5.** (A) Normalized UV melting curves for 10 μM G-quadruplex (▲), 10 μM G-quadruplex + 10 μM [Ru(bpy)2(dppz)]^{2+} in Na\(^+\) buffer (pH 5.5); 10 μM G-quadruplex (▲), 10 μM G-quadruplex + 10 μM [Ru(bpy)2(dppz)]^{2+} (▲) in K\(^+\) buffer (pH 5.5) (B) Normalized UV melting curves for 10 μM i-motif (▲), 10 μM i-motif + 10 μM [Ru(bpy)2(dppz)]^{2+} (▲) in Na\(^+\) buffer (pH 5.5); 10 μM i-motif + 10 μM [Ru(bpy)2(dppz)]^{2+} (▲) in K\(^+\) buffer (pH 5.5). The stability of G-quadruplexes DNA and i-motif DNA was assessed by UV absorbance at 295 nm and 260 nm respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Fig. 6.** Results of competition dialysis experiment. 1 μM solution of [Ru(bpy)2(dppz)]^{2+} was dialyzed against five different nucleic acid structures (10 μM) for 24 h in a buffer of 100 mM KCl, 10 mM KH_2PO_4/K_2HPO_4, 1 mM K_2EDTA (pH 5.5) respectively. The amount of [Ru(bpy)2(dppz)]^{2+} bound to each structure was plotted as a bar graph.