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Combining a Ru(II) “Building Block” and Rapid Screening Approach to Identify DNA Structure-Selective “Light Switch” Compounds

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Abstract

A chemically reactive Ru(II) “building block”, able to undergo condensation reactions with substituted diamines, was utilized to create a small library of luminescent “light switch” dipyrrodo-[3,2-*a*:2',3'-*c*] phenazine (dppz) complexes. The impact of substituent identity, position, and the number of substituents on the light switch effect was investigated. An unbiased, parallel screening approach was used to evaluate the selectivity of the compounds for a variety of different biomolecules, including protein, nucleosides, single stranded DNA, duplex DNA, triplex DNA, and G-quadruplex DNA. Combining these two approaches allowed for the identification of hit molecules that showed different selectivities for biologically relevant DNA structures, particularly triplex and quadruplex DNA.

Introduction

Small molecules have been utilized with great success to investigate DNA sequences and structures.¹ Typical interactions that may result in specific sequence and structure recognition include groove binding^{2, 3} and intercalation.⁴ Metal *complexes*^{4, 5} have been reported to demonstrate preferential binding for different types of DNA, such as duplex⁶ and G-quadruplex DNA.^{7–9} Similarly, organic molecules including cationic porphyrins have been shown to interact with G-quadruplex DNA and exhibit specific recognition of sequential or multimeric G-quadruplexes.^{10, 11} Compounds have also been designed to recognize different DNA sequences, including DNA defects, such as mismatch DNA^{12–15} and abasic sites.¹³ Certain DNA defects and structures are more prevalent in human diseases, and the design of molecules with increasingly selective DNA recognition is an active area in drug discovery research.^{7, 16–21} Two important goals involve identifying molecules that luminesce upon binding to specific DNA sequences or structures^{22–26} and assay development for rapid identification of compounds that selectively bind biologically relevant DNA targets.²⁷

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Notes

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Ruthenium (II) polypyridyl complexes have been extensively studied for biological applications, and specific compound are able to detect defects in duplex DNA, such as DNA mismatches or abasic sites,^{6, 12–15} while others have also been shown to have selectivity for G-quadruplex structures.^{28–39} Many of these complexes incorporate an imidazophenanthroline ligand that interacts with DNA through intercalation and π -stacking. Additionally, some of these compounds will induce and/or stabilize G-quadruplex structures, with potential implications for regulation of gene function or selective DNA damage.^{28–30, 33–38}

Biological sensors have been designed through the introduction of a dipyrro-[3,2-*a*:2',3'-*c*] phenazine (dppz) ligand which creates a light switch effect when bound to DNA. This behavior is a manifestation of the differential population of two triplet metal-to-ligand charge transfer (³MLCT) excited states associated with the dppz ligand, which dictates the luminescent behavior of the Ru(II) complex.^{32, 40–42} The ³MLCT states are spatially isolated on different portions of the dppz ligand; when the excited state is localized on the phenazine portion of dppz the complex is in the “dark” state and relaxes through non-radiative decay pathways. Alternatively, the complex is in the “bright” state when the ³MLCT is localized on the bipyridine (bpy) portion, and relaxes through radiative processes. In an aqueous environment, the phenazine nitrogens are involved in hydrogen bonding, lowering the energy of the “dark” ³MLCT and enhancing its population. Alternatively, when the dppz ligand is intercalated into the base stack of DNA and shielded from this hydrogen bonding, the “bright” ³MLCT state is populated and the complex is luminescent.

In an earlier study, we found [Ru(bpy)₂dppz]²⁺ exhibited enhanced luminescence when bound to the mixed-hybrid telomeric G-quadruplex compared to duplex DNA. In an effort to design more selective compounds, we recently reported a derivative of [Ru(bpy)₂dppz]²⁺, which incorporated a bromo substituent at the 11-position of the dppz ligand, [Ru(bpy)₂dppz-11-Br]²⁺ (**3**). The complex was initially screened against 32 different biomolecules, including protein, nucleosides, duplex DNA, triplex DNA, G-quadruplex DNA, and DNA containing mismatches, abasic site, or bulges. This screening revealed compound **3** exhibited enhanced selectivity for an intermolecular G-quadruplex.⁴³

In this report, we expanded upon this rapid screening approach to simultaneously evaluate the luminescent enhancement and selectivity of a small library of Ru(II) complex for different biologically relevant biomolecules and DNA sequences and structures. This provided an unbiased approach to allow for the interpretation of structure activity relationships (SAR). Such parallel screening of compounds against many potential targets is, to the best of our knowledge, lacking in the literature for metal complexes that are reported to be selective for specific DNA sequences or structures. Rather, others studies in the field rely on select sequences or structures, and experiments are designed to relate directly and exclusively to the intended target, which can introduce unintended bias and inaccuracy.

To facilitate the identification of new structure activity relationships, we chose to investigate a small collection of complexes where minor structural changes were incorporated in a systematic fashion. A Ru(II) “building block” was utilized for creation of dppz derivatives in a single step.⁴⁴ The goal was for this library of luminescent light switch derivatives to be

used with the parallel screening approach to better understand the role of the substituent identity, the substituent position, and number of substituents on the selectivity for different DNA structures.

Results and Discussion

A library of luminescent light switch derivatives was synthesized through a condensation reaction of $[\text{Ru}(\text{bpy})_2\text{phenanthroline}]^{2+}$, the Ru(II) building block, with halogen substituted α -phenylenediamines (Figure 1). As demonstrated by Hartshorn and Barton,⁴⁴ the Ru(II) complex is a synthetic intermediate that provides for the convenient creation of dppz derivatives in a single step. The complexes were purified via flash chromatography and obtained in 60–82% yield.

In order to identify compounds that exhibited selectivity for different biological molecules, a rapid screening approach was used. A variety of systems were explored, including bovine serum albumin (BSA), nucleosides, single strand DNA, duplex DNA, duplex DNA containing mismatches, abasic sites, and bulges, triplex DNA, and G-quadruplex DNA.⁴³ The assay was designed to test the impact of a variety of factors on light switch response in parallel. The questions we posed included: 1) is the luminescent enhancement of the complexes only dependent on a hydrophobic environment? 2) do the number of DNA strands/ π stacking surface area directly correlate to the luminescence? 3) does the nucleic acid base composition matter? 4) does the specific three-dimensional structure have an impact? 5) do sequential G-quadruplexes enhance the luminescence response?

To answer these questions, the response of compounds **1–9** was examined by measuring full spectrum luminescence in the presence of 38 different biomolecules. This assay was designed and optimized using 384-well plates to minimize the material needed. As the luminescence response, not the binding affinity, is the first and most important property to characterize in the identification of useful fluorescent reporters, this study focuses on this feature. Accordingly, each complex was screened under saturation conditions. The Ru(II) complexes were incubated at a 1:5 ratio of $[\text{Ru}]:[\text{biomolecule}]$ for nucleosides and BSA. For the DNA studies, the concentration of DNA was determined as [nucleotide phosphate] (NP); the $[\text{Ru}]:[\text{NP}]$ was 1:5 for single strand and G-quadruplex DNA, 1:10 for double strand DNA, and 1:15 for triplex DNA. The work was done under saturating conditions to ensure the responses could be directly compared regardless of sequence; this allows for the direct comparison of all compounds independent of the Ru(II) complex binding affinities for the different biomolecules.⁴⁵

To confirm that experiments were conducted under saturating conditions, binding titrations of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ and **3** were performed, and exhibited saturation around a 1:4 $[\text{Ru}]:[\text{NP}]$ ratio.⁴³ The addition of the 11-Br substituent in compound **3** lowers the binding affinity to the G-quadruplexes, Seq27 and Seq30, by ~2.5-fold.⁴³ Additionally, the binding stoichiometries of $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ and **3** were >1:1 when bound to the G-quadruplexes, and the broad Job plots of **3** suggest that multiple binding events or modes were occurring.⁴³ For the initial study, we hypothesized that the sensitivity of the complexes is dominated by

the binding orientation when bound to the different DNA sequences and structures, not by a major shift in binding affinity.⁴³

In order to test if each of the Ru(II) complex's luminescent response was selective for nucleic acids rather than any hydrophobic environment, BSA was investigated, as BSA is commonly used as a model hydrophobic protein. In addition, each individual nucleoside was tested to confirm the requirement of polymeric nucleic acid structures for the light switch effect. The DNA sequences and structures that were studied were designed to determine the importance of base composition and number of DNA strands present. Base composition was also examined through the use of different single strand and double strand DNA sequences (Seq 1–11), specifically, poly G, poly C, poly GC, poly A, poly T, and poly AT sequences. Damaged DNA was tested, including mismatches, abasic sites and bulges. Single, double, triplex and G-quadruplex structures were used to determine the impact of increasing the number of strands and the associated π surface area on the luminescence response. Lastly, different G-quadruplex structures were studied to determine selectivity for the particular three-dimensional fold.

To investigate sensitivity to subtle differences in the three-dimensional folding, 7 different G-quadruplex structures and 2 corresponding G-triplexes were examined. The biological significance of the G-quadruplexes chosen for the study stem from either forming from the human telomeric sequence or from the promoter region of *c-myc*, an oncogene. The different telomeric structures include the mixed-hybrid 1/2 (Seq30), antiparallel basket (Seq29), mixed-hybrid 1 (Seq32), and mixed-hybrid 2 (Seq31). The i-motif (Seq25), the complementary sequence for the telomeric G-quadruplexes (Seq29 and Seq30), was also tested, along with a variant of this sequence where the TAA sequence was substituted with TTA (seq26). In addition, a double G-quadruplex (Seq33) was used to better mimic telomeres, which contain repeating G-quadruplex structures connected by a short, 3 nucleotide strand that forms a cleft between the two folded tetrads.⁴⁶ The double quadruplex contained a sequence with 8 repeating G-rich regions that has a near identical structure to Seq30 (as seen in the CD spectra in Figure S9). Thus, the primary difference between the two structures is the presence of the cleft between the two quadruplexes.

Effect of hydrophobic environment

Most of the complexes were non-luminescent in the buffer solution, which was used as a control. However, compounds **1**, **2**, and **4** showed slight luminescence in buffer. None of the complexes showed a significant luminescence enhancement in the presence of BSA, indicating that the hydrophobic surfaces provided by this protein are not sufficient to induce switching of the excited states. Indeed, the parent unsubstituted dppz complex exhibited the largest enhancement in the presence of BSA (4.5-fold compared to buffer). Only the fluorinated compound **1** gave a response of similar scale (3.4-fold), demonstrating that all other modifications actually reduced the emission response with the hydrophobic protein. Individual nucleosides also were not able to induce emission.

Importance of number of DNA strands

All of the complexes exhibited enhanced luminescence responses in the presence of DNA (Figure 2 and Figures S1–3). A common trend emerged where increasing the number of DNA strands increased the brightness of **1–9** (Figure S4). While single stranded DNA induced an average fold change in emission of 7.1x, relative to buffer, duplex (11.8x), triplex (29.6x) and quadruplex (44.7x) DNA structures were sequentially brighter. Only compound **3** was an outlier in that it was more emissive in the presence of single stranded DNA (average of 12.8x) than duplex (average of 7.7x). The emission behavior was also dependent on DNA sequence.

Figure S4 correlates the luminescence intensity of each of the 9 dppz compounds to the number of DNA strands. Graphs A–C reflect the importance of the halogen identity (F, Cl, Br) at a single position, and graphs D–F represent the impact of the substituent position and number for each halogen. The identity of the halogen is important for the overall behavior when comparing the luminescence to the number of DNA strands. This is evident in the differences in the slopes of each data set (Figure S4 A–C and Table S2), where the data set is defined as the average luminescence area of a compound with the various DNA sequences (single, double, triple or quadruple stranded) versus the number of DNA strands, and the slope represents the change in luminescence as the number of DNA strands increases. When the substituent is F or Cl, the position or the number of substituents does not seem to matter, as apparent by the overlapping data sets (Figure S4 D, E).

Both the position and number of Br substituent(s) is important for the average response in relation to the number of DNA strands. Compound **6**, 10-Br, gives the largest degree of change (the greatest slope). In contrast, compound **9**, 11,12-Br, shows the shallowest slope, due to the minimal enhancement when bound to G-quadruplexes versus single or double strand DNA. Interestingly, the Br substituted compounds for each series show the lowest R^2 value for improved luminescence to increasing DNA strands (Table S2). This can be attributed to the fact that brominated compounds **3**, **6**, and **9** have a large signal increase when bound to higher order structures, but this increase is variable and highly dependent on the specific quadruplex structure.

To further explore the selectivity for particular structures, the standard deviation was calculated for each molecule when associated with single stranded, duplex, triplex or quadruplex DNA, where the standard deviation represents the variability of the luminescence response within a given number of DNA strands (Figure S5). The luminescence response of each of the compounds in single, double, and triple strand DNA showed low standard deviations, ranging only from 0.1–9%. Conversely, the variation in luminescent response ranges from 15–43% when in the presence of the G-quadruplex structures. Thus, the variability in signal response for each set of DNA strand allows for rapid identification of compounds that are highly structure selective. A small variation in signal indicates that the complex has little to no selectivity for one sequence over another for DNA with equal number of strands. In contrast, a large variation shows the complex has some selectivity for one or two specific structures (or structural features) over the others. The high variation in luminescent response in the presence of the G-quadruplex DNA is

likely due to the drastic differences in how each G-rich sequence folds into its respective G-quadruplex structure.

Figure 3 shows the luminescence fold change of each complex when bound to selected triplex or G-quadruplex DNA as compared to the luminescence when bound to duplex DNA (CT DNA). The results indicate that many of the substituted compounds have a distinct preference for specific DNA structures to a greater extent than the parent complex, $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$, as evident by the larger fold changes. The 11,12-substituted compounds, **7–9**, exhibited the greatest differences amongst the triplex sequences, where **9** had a significant preference for Seq22 ($>3.5\times$). Compounds **1–6** exhibited lower luminescence fold changes when bound to the different triplex structures tested, but the enhancements were greater than with the parent dppz complex.

The selectivity of compound **9** for Seq22 must be related to the DNA structure rather than the sequence, as little luminescence enhancement was found with poly A poly T (Seq9) and poly AT (Seq10) duplexes. In fact, the enhancement in the T-A-T triplex was $34\times$ greater than in the two AT-rich duplexes (Seq9 and Seq10). We hypothesize that the two large Br groups at the 11- and 12-positions of **9** prevent full intercalation into duplex DNA, causing the phenazine nitrogens to be partially water exposed, resulting in only a $2.6\text{--}3.6\times$ luminescence enhancement as compared to buffer. In contrast, the larger surface area of the triplex Seq22 aids in shielding the phenazine nitrogens, allowing for $89\times$ luminescence enhancement when compared to buffer. This hypothesis is further supported by the behavior of compounds **7** and **8**. Compound **7**, with two small fluorine substituents at the 11- and 12-positions has only a slight luminescence enhancement (average of $2.5\times$) in the Seq22 compared to the AT rich duplexes Seq9 and Seq10. Increasing the size of the substituents to chlorines in compound **8** slightly increased the luminescence difference in the Seq22 compared to the AT rich duplexes, with a $3.2\times$ enhancement.

In addition to size, the polarizability of the halogen substituents may be important for the behavior of these complexes in the presence of duplex, triplex and G-quadruplex DNA. Highly polarizable atoms increase the London dispersion interactions and therefore could have an enhanced interaction with the π system of the DNA base stack. A trend relating to polarizability was observed for the 11-substituted complexes. The fluorine substituent has the lowest polarizability (3.76 a.u.)⁴⁷ and exhibited the smallest luminescence enhancement as the number of DNA strands increased, whereas the bromine substituents are highly polarizable (21.9 a.u.)⁴⁸ and gave the largest luminescence enhancement when increasing the number of DNA strands, with a maximum of 18.4 for the ratio of emission in G-quadruplex Seq27 to CT DNA. To distinguish if polarizability is a more important feature than size, $[\text{Ru}(\text{bpy})_2\text{dppz-11-CH}_3]^{2+}$ was tested. The CH_3 group is a non-polarizing substituent with a similar size to the Br substituent, with a Van der Waals radius of $1.95\text{ \AA}$ (Br) versus $2.0\text{ \AA}$ (CH_3).⁴⁹ The addition of an 11- CH_3 substituent results in a complex that has little selectivity for G-quadruplex DNA; there is a 62 ± 12 -fold luminescence enhancement in the presence of duplex DNA over buffer vs an 84 ± 37 -fold luminescence enhancement in the presence of G-quadruplex DNA, for a ratio of only 1.3. This data suggests that polarizability may be an important feature for designing complexes that have selectivity for G-quadruplex DNA over duplex DNA.

Many of the substituted complexes showed varying selectivity for G-quadruplex DNA (Figure 3 and Figures S1–3). The most selective compound was **9**, which exhibited the largest luminescence increase when bound to the parallel intermolecular G-quadruplex (Seq27; 64-fold luminescence enhancement compared to duplex DNA). Generally, the highest luminescence response was observed for the Seq27, as exhibited by compounds **1–3**, **7**, and **9**. A 1.2-fold increase over other G-quadruplex DNA sequences was observed, with a 14–160-fold change over buffer alone. This selective response was not observed for either $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ or $[\text{Ru}(\text{bpy})_2\text{dppz-11-CH}_3]^{2+}$, highlighting the importance of the nature of the substituents. Surprisingly, **4** exhibited slight selectivity for the variant of the i-motif (Seq26), and **8** had slight selectivity for Seq31.

Impact of nucleic acid base composition

Since complexes **1–9** have an overall higher luminescence response when associated with G-quadruplex DNA, we tried to distinguish between the impacts of base composition vs. specific topology by correlating the luminescence area to the number of guanosines (Figure S6). The sequences analyzed were (Seq1 (with 2 Gs), Seq2 (6 Gs), Seq23 (9 Gs), Seq32 (12 Gs), and Seq33 (24 Gs) (Table S1)). The parent complex, $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$, and the 10-substituted series exhibited a near linear correlation ($R^2 > 0.95$) between emission enhancement and the number of guanosines in the DNA sequences. Additionally, the slope (luminescence area versus number of guanosines) of the 10-substituted series resulted in a linear correlation to the atomic diameter of the halogen substituent ($R^2 = 0.94$, Figure S7). In contrast, the 11-substituted series and the 11, 12-substituted series ranged from $R^2 = 0.40$ – 0.92 and no clear correlation was present (Table S3). This suggests that substituents at the 11- and 12-positions do not necessarily dictate how the dppz ligand is binding to the different DNA structures.

The 10-position is important, as substituents at this position can shield the phenazine nitrogen. In addition, recent crystal structures of $[\text{Ru}(\text{phen})_2\text{dppz}]^{2+}$, $[\text{Ru}(\text{bpy})_2\text{dppz}]^{2+}$ and $[\text{Ru}(\text{TAP})_2\text{dppz-10-CH}_3]^{2+}$ (TAP = 1,4,5,8-tetraazaphenanthrene), demonstrated that the angle of intercalation changed with the addition of the 10-CH₃ substituent.^{12, 50, 51} The unsubstituted complexes bind DNA with two modes of intercalation: symmetrically perpendicular and angled. The symmetric intercalation occurs at a AT step and the phenazine nitrogens were able to hydrogen bond with water molecules, whereas the angled intercalation occurs at a GC step and prevented hydrogen bonding to one of the phenazine nitrogens.⁵⁰ The addition of a 10-CH₃ substituent to the dppz ligand results in an angled intercalation mode at a TC/AG step and prevented the phenazine nitrogen on the non-methylated side from hydrogen bonding with water. Additionally, the angled intercalation caused by the 10-CH₃ group prevents the adjacent phenazine nitrogen from hydrogen bonding.⁵¹ Given this structural information, we hypothesize that the size of the substituent at the 10-position is extremely important for how the dppz ligand binds to the different DNA structures, and in turn, the extent of luminescence enhancement.

In addition to increasing the number of guanosines, we also examined different damaged DNA duplexes, such as mismatched bases, base bulges, and abasic sites. Unfortunately, none

of the complexes showed noteworthy emission enhancement in these sequences over the parent duplex sequence.

Impact of three-dimensional structure

As the luminescence of the complexes increased with the number of DNA strands and the number of guanines in the DNA sequence, further analysis was performed to determine if the complexes were able to detect differences in structure and folding direction. Initially, we looked at the luminescence response with two G-quadruplexes and their analogous triplexes (Figure 4). G-rich triplexes have been shown to form under physiological conditions, allowing for an intermediate 3 dimensional structure that lacks the fourth G track to form a G-quadruplex.⁵² The G-quadruplexes have the same G-rich repeating sequence but differ by two bases at both the 5' and 3' ends of the sequence. Substituting the two 5' and two 3' adenosines for thymidines inverts the folding direction of the G-quadruplex.^{53, 54} Since the only base composition difference is at the 3' or 5' end of the triplexes versus the G-quadruplexes in this case, the analysis is solely driven by structural differences, rather than sequence changes.

The solution structures of Seq32 and Seq31 sequences have been previously reported as the hybrid 1 and hybrid 2 G-quadruplexes, respectively.^{54, 55} Additionally, it has been proposed that substitution of one set of guanines with thymidines on either the 5' end of Seq32 or 3' end of Seq31 results in the G-triplex folding intermediate.⁵⁶ The analogous structural features of the Seq32 and Seq31 were visualized by the similarities in the CD spectra (Figure 4F). Similarly, the two triplex sequences have near identical CD signals. However, comparison of the Seq32 versus Seq23 and Seq31 versus Seq24 demonstrated that there are notable differences in their CD spectra. These structural differences allow for the Ru(II) complexes to differentiate the G-quadruplex from the G-triplex. In general, the complexes have greater luminescence enhancement when bound to a G-quadruplex over a G-triplex, with up to a 2.8x increase in luminescence. The preference for the G-quadruplex over the G-triplex suggests that it is either the presence of a G-tetrad or the specific loop region that is unfolded in the G-triplex that is essential for the response of **1–9**.

As expected given the similarity in the structure, there was not a major preference for the hybrid 1 versus hybrid 2 structures or the 5'-triplex versus the 3'-triplex in reference to the number of halogens, the halogen identity, or the position. Indeed, **6** had the highest fold change, which was only 1.7x when bound to Seq32 over Seq31 (Figure S8). Similarly, **9** had the largest luminescence enhancement of 1.4x when bound to the Seq23 over the Seq24 (Figure S8).

Additionally, we analyzed the data for three different G-quadruplex structures with similar sequences and folded in the same buffer, where each of the G-quadruplexes is associated with K⁺ ions. Seq30, a mixture of the hybrid 1 and hybrid 2 structures,⁵⁷ is compared to Seq31 (hybrid 2) and Seq32 (hybrid 1). The structures, by CD, have major differences at 270 nm, where the hybrid 1 structure has a larger positive ellipticity than the other sequences, and Seq30 has the lowest positive ellipticity (Figure 4H). As none of the complexes showed significant preference for the hybrid 1 or hybrid 2 structures, we expected the complexes to have a similar light switch response with Seq30 as the average response from Seq32 and

Seq31. All complexes exhibited luminescence with the Seq30 within 2-fold of the average luminescence of the hybrid 1 and hybrid 2 G-quadruplexes (Figure 4G).

Next, we screened each complex against four G-quadruplexes that fold into quite different structures. The four structures examined were the parallel intermolecular G-quadruplex (Seq27), the parallel propeller G-quadruplex (Seq28), the antiparallel basket telomeric G-quadruplex (Seq29), and the mixed-hybrid telomeric G-quadruplex (Seq30). The major differences between the four G-quadruplexes are the presence and positioning of loop regions which affects the accessibility of the G-tetrad. In recent reports, the loop regions have been essential for the binding of certain metal complexes, specifically Zn^{2+} , where they interact with the thymidines within the loop region rather than with the G-tetrad.^{58–60} We hypothesize that the loop regions are also important for recognition by the Ru(II) dppz complexes by either preventing binding to the G-tetrad or by shielding the phenazine nitrogens. Seq27 lacks loops, whereas Seq28–Seq30 each have 3 loop regions (see Figure 7). For Seq27, the G-tetrad is readily accessible and the dppz ligands can easily π stack within one G-quadruplex or between two G-quadruplex. The parallel propeller folding of Seq28 results in tight loops spanning each G-tetrad on 3 of 4 sides of the G-quadruplex. The placement of these loops significantly restricts the access of the dppz to π stack within one G-quadruplex, but the top and bottom face do not have loops so the dppz ligand can π stack between two different G-quadruplexes. For Seq30, the loops are placed in a way that the dppz ligand would likely bind by threading through one of the lateral loops and π stack as an end cap. Likewise, Seq29 has lateral loops and a diagonal loop that the dppz ligand has to thread through to π stack with one of the end G-tetrad.

Interestingly, none of the complexes have a large luminescence response with Seq29. In fact, they only show a maximum of an 8.4-fold enhancement in comparison to CT DNA. As an end-capping mode of binding is likely for these dppz complexes, the diagonal loop may be in the wrong orientation to effectively shield both phenazine nitrogens, leaving the complex in the dark state. This would account for the minimal luminescent enhancement observed.

The 10-substituted complexes, **4**, **5**, and **6**, do not have a strong preference for any of the 4 G-quadruplexes (Figure 5). Instead, this series of complexes has some selectivity for either the mutated i-motif (**4**) or the double G-quadruplex (**5** and **6**). The 11-substituted and 11, 12-substituted series prefer the inter G-quadruplex. The number of substituents is important for the degree of selectivity for the inter G-quadruplex (Figure 5). The 11-substituted series shows a higher luminescence bound to the inter G-quadruplex when the substituent is either Cl or Br. On the other hand, for the 11, 12-substituted series, the F-substituted complex gives a larger luminescence when bound to the inter G-quadruplex than Cl or Br substituents. The position is also important for the selectivity, as the 10-substituted series do not have a preference for the inter G-quadruplex.

When comparing the luminescence response of each complex when bound to the inter G-quadruplex vs duplex DNA, the larger substituents (Cl and Br) consistently have a greater fold change than the F substituent. This is likely due to the increase in size, causing complexes with the Cl and Br substituents to be more sensitive to the increased π surface. Similarly, the 11,12-substituted series has an enhanced response over the 11- and 10-

substituted series. The double substitution may force the dppz into one orientation when bound to the inter G-quadruplex that prevents either phenazine nitrogen from being exposed to water, increasing the luminescence.

Impact of sequential G-quadruplexes

Lastly, we were interested in the impact sequential G-quadruplexes have on the luminescence response. Seq30 (a single quadruplex) and Seq33 (the double G-quadruplex) have near identical CD spectra, confirming they both have a mixture of the hybrid 1 and hybrid 2 folding (Figure S9). Seq33 has 2 G-quadruplexes separated by a short 3 nucleotide sequence, introducing 2x the binding sites as well as a cleft region between the G-quadruplexes. Since the experiments were completed using equal [Ru] and identical [Ru]:[DNA (NP)], the difference in the number of binding sites between Seq30 and Seq33 is not a contributing factor to the response.

When comparing the response of each compound when bound to the double G-quadruplex DNA, **5** and **6** showed pronounced selectivity (Figure 6). Compound **5** and **6** exhibited 46-fold and 64-fold luminescence enhancement when compared to CT DNA, and were 4.8 and 4.2x more selective for Seq33 over Seq30. Given these results, we hypothesize that the >4x enhancement is due to selective binding orientation with Seq33, where the cleft region between quadruplexes may be important, rather than the increased number of binding sites, as there are only twice the potential sites in Seq33 vs. Seq30. Complexes **5** and **6** are thus the most biologically relevant hit complexes for future *in vivo* applications where repeating G-quadruplexes are prevalent, *i.e.* in telomeres.

Conclusions

A series of halogenated dppz Ru(II) complexes were synthesized using a Ru(II) “building block” containing reactive functional groups. This series was investigated to reveal the importance of the substituent identity, the substituent position, and the number of substituents on “light switching” ability. Each dppz derivative was tested using a rapid, unbiased screening approach to determine the luminescence response against a series of biomolecules. The biomolecules tested were selected to probe biologically relevant DNA sequences and structures. In addition, the impact of fundamental features of nucleic acid structure were investigated, and the size of the π surface emerged as a more important factor than specific sequences or damage sites.

A few conclusions may be drawn from this study. First, unlike DNA, the hydrophobic protein BSA is not able to induce large increases in luminescence with these compounds. Second, there is a general trend towards increasing emission with increasing number of DNA strands, though compound **3** was found to be an exception to this rule. Expansion of the π surface is a very important feature, more so than sequence, though DNA sequence also was a factor, as reflected by the relationship between luminescence enhancement and the number of guanosine residues. This was found to depend on the size of the substituents, but only for those located at the 10-position. The folding of the DNA was found to play a major role in the luminescence enhancement, with different compound structures exhibiting different selectivity profiles. It must be stressed, however, that all these studies were

conducted with racemic mixtures, and it is anticipated that the Δ and Λ enantiomers will have different binding affinities and luminescent enhancements with these three-dimensional DNA structures. Such studies would be worth pursuing with the complexes which gave the best responses and selectivities in this screen.

There are many Ru(II) complexes that have been identified as exhibiting “light switch” behavior with DNA. However, there is a lack of systematic studies in the literature on either the impact of subtle structural modifications to the metal complex or real attempts to investigate structural selectivity by comparing in parallel many nucleic acid sequences and structures. We feel both approaches are needed to first identify structure activity relationships, and to then rationally design optimized probes. Indeed, the combination of both approaches in this report allowed for the rapid identification of hit compounds that can be improved for future biological applications, with the emergence of several unanticipated structure-selectivity relationships. Compound **9** exhibited selectivity for T-A-T triplex DNA and interstrand G-quadruplex DNA. Compounds **5** and **6** showed the greatest selectivity for the double G-quadruplex, a more biologically relevant mimic of the G-quadruplexes formed in telomeres. These two complexes not only had the greatest luminescence area but they also had the largest fold change over duplex DNA, and may prove to be important for detection of G-quadruplexes *in vivo*. This study reveals that surprisingly small changes in the substituents on dppz ligands have a large impact on luminescent response, and further, that the position and number of substituents helps to drive the selectivity for triplex vs. quadruplex DNA.

Experimental Section

Materials and instrumentation

All chemicals were of reagent grade and were used without further purification. *cis*-Dichlorobis(2, 2'-bipyridine)ruthenium(II) dihydrate was purchased from Strem Chemicals. Custom DNA sequences were purchased from Eurofins. All ^1H -NMR and ^{13}C -NMR were obtained on a Varian Mercury spectrometer (400, 100 MHz). The ^1H chemical shifts are reported relative to the residual solvent peak of CD_3CN at δ 1.94. The ^{13}C chemical shifts are referenced to CD_3CN at δ 1.39. Electrospray ionization (ESI) mass spectra were obtained on a Varian 1200L mass spectrometer at the Environmental Research Training Laboratory (ERTL) at the University of Kentucky. Luminescence spectra were obtained on a Molecular Devices Spectramax M5 microplate reader. Circular dichroism (CD) experiments were performed on a Jasco J-815 CD Spectrometer equipped with an MPTC-490S/15 temperature controller. HPLC experiments were run on an Agilent 1100 Series HPLC equipped with a model G1311A quaternary pump, G1315B UV diode array detector and Chemstation software version B.01.03. Chromatographic conditions were optimized on a Column Technologies Inc. C18 120 Å column fitted with a Phenomenex C18 guard column. The Prism software package was used to analyze kinetic data with a single exponential equation and luminescence spectra with area under the curve.

Synthesis

Note: the numbering for phen and dppz ligands is different. The 2,9 positions on phen are *ortho* to the pyridyl nitrogens, analogous to the 3,6 positions on dppz.

1,10-Phenanthroline-5,6-dione (phendione) and $[\text{Ru}(\text{bpy})_2\text{phendione}](\text{PF}_6)_2$ were prepared as previously reported.^{43, 44} The synthesis of $[\text{Ru}(\text{bpy})_2\text{dppz-11-Br}]^{2+}$ (**3**) was previously reported.⁴³

General Synthesis of 1, 2 and 3–9: All complexes were prepared in a similar manner. The synthesis of compound **1** is shown as an illustrative example. A solution of $[\text{Ru}(\text{bpy})_2\text{phendione}](\text{PF}_6)_2$ (120 mg, 0.131 mmol) and 4-fluoro-*o*-phenylenediamine (18 mg, 0.100 mmol) in ethanol (8 mL) was refluxed at 80 °C for 1 h. The resulting red mixture was cooled to room temperature, diluted with water, filtered and washed with water and ether. Purification by flash chromatography (silica, eluting at 90:10:0.1 acetonitrile/water/saturated KNO_3) gave the pure product. The solvent was removed under vacuum, 5 mL of dd H_2O was added, and the complex was converted to PF_6^- salt by addition of a saturated solution of KPF_6 . The complex was extracted into CH_2Cl_2 and the solvent removed under vacuum to give a red solid.

$[\text{Ru}(\text{bpy})_2\text{dppz-11-F}](\text{PF}_6)_2$ (1**)**—The product was obtained in 77% yield (102 mg) as a red solid. ^1H NMR (CD_3CN , 400 MHz): δ 9.61 (d, J = 8.2 Hz, 2H), 8.69 (s, 2H), 8.58 (d, J = 8.2 Hz, 2H), 8.55 (d, J = 8.2 Hz, 2H), 8.21 (dd, J = 7.8, 1.2 Hz, 2H), 8.14 (td, J = 7.8, 1.6 Hz, 2H), 8.05 (td, J = 8.2, 1.6 Hz, 2H), 7.92 (dd, J = 8.2, 5.5 Hz, 2H), 7.87 (d, J = 5.5 Hz, 2H), 7.75 (d, J = 5.5 Hz, 2H), 7.49 (ddd, J = 7.2, 5.9, 1.2 Hz, 2H), 7.29 (ddd, J = 7.2, 5.9, 1.2 Hz, 2H). ^{13}C NMR (CD_3CN , 100 MHz): δ 158.23, 158.03, 155.23, 153.22, 153.04, 151.81, 142.45, 142.03, 139.10, 139.01, 137.50, 134.64, 131.47, 131.24, 128.71, 128.67, 128.56, 125.42, 125.36; Purity by HPLC: 98.2% by area. UV/Vis in CH_3CN , λ_{max} nm (ϵ $\text{M}^{-1} \text{cm}^{-1}$): 280 (109,200), 380 (23,400), 440 (18,200). ESI MS calcd for $\text{C}_{38}\text{H}_{25}\text{FN}_8\text{Ru}$: $[\text{M}^{2+} \cdot \text{PF}_6^-]^+$ 859.08, $[\text{M}]^{2+}$ 357.06, found 858.4 $[\text{M}^{2+} \cdot \text{PF}_6^-]^+$, 356.6 $[\text{M}]^{2+}$.

$[\text{Ru}(\text{bpy})_2\text{dppz-11-Cl}](\text{PF}_6)_2$ (2**)**—The product was obtained in 69% yield (93 mg) as a red solid. ^1H NMR (CD_3CN , 400 MHz): δ 9.64 (ddd, J = 8.2, 4.7, 1.2 Hz, 2H), 8.58–8.46 (m, 6H), 8.22–8.19 (m, 2H), 8.16–8.09 (m, 3H), 8.05 (td, J = 7.8, 1.6 Hz, 2H), 7.92 (ddd, J = 8.2, 5.5, 2.0 Hz, 2H), 7.88–7.86 (m, 2H), 7.76–7.74 (m, 2H), 7.50 (ddd, J = 7.2, 5.9, 1.2 Hz, 2H), 7.29 (ddd, 7.2, 5.9, 1.2 Hz, 2H). ^{13}C NMR (CD_3CN , 100 MHz): δ 158.29, 158.09, 155.13, 155.00, 153.24, 153.08, 151.81, 151.63, 143.91, 142.45, 141.84, 141.32, 139.12, 139.04, 138.96, 134.67, 134.60, 134.35, 132.35, 131.73, 131.63, 129.27, 128.75, 128.62, 128.61, 125.46, 125.40; Purity by HPLC: 96.9% by area. UV/Vis in CH_3CN , λ_{max} nm (ϵ $\text{M}^{-1} \text{cm}^{-1}$): 280 (103,500), 365 (19,200), 440 (18,000). ESI MS calcd for $\text{C}_{38}\text{H}_{25}\text{ClN}_8\text{Ru}$: $[\text{M}^{2+} \cdot \text{PF}_6^-]^+$ 875.05, $[\text{M}]^{2+}$ 365.05, found 857.1 $[\text{M}^{2+} \cdot \text{PF}_6^-]^+$, 364.6 $[\text{M}]^{2+}$.

$[\text{Ru}(\text{bpy})_2\text{dppz-10-F}](\text{PF}_6)_2$ (4**).**^{61,62}—The product was obtained in 60% yield (79 mg) as a red solid. ^1H NMR (CD_3CN , 400 MHz): δ 9.65 (dt, J = 8.2, 1.6 Hz, 2H), 8.56 (d, J = 8.2, 2H), 8.53 (d, J = 8.2, 2H), 8.32 (dt, J = 8.6, 0.8 Hz, 1H), 8.20 (dt, J = 5.5, 1.6 Hz, 2H), 8.15–8.08 (m, 3H), 8.03 (td, J = 7.8, 1.6 Hz, 2H), 7.90 (ddd, J = 8.4, 5.3, 3.1 Hz, 3H), 7.87–7.83 (m, 2H), 7.74 (dq, J = 5.5, 0.8 Hz, 2H), 7.5 (ddd, J = 7.6, 5.7, 1.2 Hz, 2H), 7.27 (ddd, J

= 7.9, 5.9, 1.2 Hz, 2H). ^{13}C NMR (CD_3CN , 100 MHz): δ 159.47, 158.26, 158.06, 156.89, 155.16, 155.10, 153.22, 153.07, 151.84, 151.75, 144.50, 141.87, 140.96, 139.10, 139.02, 134.69, 133.16, 133.08, 131.71, 131.65, 128.72, 128.63, 128.57, 126.74, 126.70, 125.43, 125.38, 116.78, 116.59; Purity by HPLC: 99.9% by area. UV/Vis in CH_3CN , λ_{max} nm ($\epsilon \text{ M}^{-1} \text{ cm}^{-1}$): 285 (114,700), 360 (17,100), 440 (19,000). ESI MS calcd for $\text{C}_{38}\text{H}_{25}\text{FN}_8\text{Ru}$: $[\text{M}^{2+}\cdot\text{PF}_6^-]^+$ 859.08, $[\text{M}]^{2+}$ 357.06, found 859.0 $[\text{M}^{2+}\cdot\text{PF}_6^-]^+$, 356.7 $[\text{M}]^{2+}$.

[Ru(bpy) $_2$ dppz-10-Cl](PF $_6$) $_2$ (5)—The product was obtained in 70% yield (94 mg) as a red solid. ^1H NMR (CD_3CN , 400 MHz): δ 9.71 (dd, J = 8.2, 1.2 Hz, 1H), 9.65 (dd, J = 8.2, 1.6 Hz, 1H), 8.58–8.52 (m, 4H), 8.44 (dd, J = 8.6, 1.2 Hz, 1H), 8.26 (dd, J = 7.4, 1.2 Hz, 1H), 8.21 (dd, J = 5.5, 0.8 Hz, 2H), 8.13 (td, J = 8.2, 1.6 Hz, 2H), 8.09–8.01 (m, 3H), 7.91 (ddd, J = 7.8, 5.9, 2.0, 2H), 7.88–7.86 (m, 2H), 7.76–7.73 (m, 2H), 7.48 (ddd, J = 7.2, 6.1, 0.8 Hz, 2H), 7.30–7.25 (m, 2H). ^{13}C NMR (CD_3CN , 100 MHz): δ 158.27, 158.07, 155.17, 155.14, 153.25, 153.20, 153.06, 151.85, 151.81, 144.68, 141.75, 141.16, 140.39, 139.10, 139.03, 134.80, 134.75, 134.16, 133.28, 133.00, 131.76, 131.51, 130.01, 128.73, 128.68, 128.65, 128.59, 125.43, 125.38; Purity by HPLC: 99.2% by area. UV/Vis in CH_3CN , λ_{max} nm ($\epsilon \text{ M}^{-1} \text{ cm}^{-1}$): 285 (93,300), 362 (15,500), 440 (16,100). ESI MS calcd for $\text{C}_{38}\text{H}_{25}\text{ClN}_8\text{Ru}$: $[\text{M}^{2+}\cdot\text{PF}_6^-]^+$ 875.05, $[\text{M}]^{2+}$ 365.05, found 874.6 $[\text{M}^{2+}\cdot\text{PF}_6^-]^+$, 364.7 $[\text{M}]^{2+}$.

[Ru(bpy) $_2$ dppz-10-Br](PF $_6$) $_2$ (6)—The product was obtained in 68% yield (80 mg) as a red solid. ^1H NMR (CD_3CN , 400 MHz): δ 9.71 (dd, J = 8.2, 1.2 Hz, 1H), 9.65 (dd, J = 8.2, 1.6 Hz, 1H), 8.57–8.52 (m, 4H), 8.47 (ddd, J = 8.0, 5.3, 1.2 Hz, 2H), 8.21 (dt, J = 5.1, 1.2 Hz, 2H), 8.15–8.11 (m, 2H), 8.05–7.99 (m, 3H), 7.93–7.89 (m, 2H), 7.87–7.85 (m, 2H), 7.76–7.73 (m, 2H), 7.48 (ddd, J = 6.7, 5.9, 0.8 Hz, 2H), 7.30–7.25 (m, 2H). ^{13}C NMR (CD_3CN , 100 MHz): δ 158.26, 158.06, 155.13, 153.25, 153.20, 153.07, 153.05, 151.86, 151.81, 144.69, 141.78, 141.41, 141.21, 139.09, 139.01, 136.68, 134.85, 134.79, 133.84, 131.70, 131.46, 130.73, 128.72, 128.64, 128.57, 125.42, 125.36, 125.13; Purity by HPLC: 99.9% by area. UV/Vis in CH_3CN , λ_{max} nm ($\epsilon \text{ M}^{-1} \text{ cm}^{-1}$): 285 (102,300), 365 (17,200), 440 (17,400). ESI MS calcd for $\text{C}_{38}\text{H}_{25}\text{BrN}_8\text{Ru}$: $[\text{M}^{2+}\cdot\text{PF}_6^-]^+$ 919.0, $[\text{M}]^{2+}$ 387.02, found 918.8 $[\text{M}^{2+}\cdot\text{PF}_6^-]^+$, 386.7 $[\text{M}]^{2+}$.

[Ru(bpy) $_2$ dppz-11,12-F](PF $_6$) $_2$ (7)—The product was obtained in 77% yield (104 mg) as a red solid. ^1H NMR (CD_3CN , 400 MHz): δ 9.63 (dd, J = 8.2, 1.2 Hz, 2H), 8.56 (d, J = 7.8 Hz, 2H), 8.52 (d, J = 7.8, 2H), 8.32 (t, J = 9.8 Hz, 2H), 8.19 (dd, J = 5.5, 1.2 Hz, 2H), 8.13 (td, J = 7.8, 1.6 Hz, 2H), 8.03 (td, J = 7.8, 1.6 Hz, 2H), 7.91 (dd, J = 8.2, 5.5 Hz, 2H), 7.87–7.85 (m, 2H), 7.72 (dq, J = 5.9, 0.8 Hz, 2H), 7.47 (ddd, J = 7.2, 5.9, 1.2 Hz, 2H), 7.27 (ddd, J = 7.8, 5.7, 1.6 Hz, 2H). ^{13}C NMR (CD_3CN , 100 MHz): δ 158.25, 158.06, 156.59, 156.40, 155.04, 154.00, 153.82, 153.20, 153.05, 151.57, 141.76, 141.70, 141.63, 141.27, 139.09, 139.00, 134.56, 131.51, 128.71, 128.59, 128.56, 125.42, 125.36, 116.14, 116.08, 116.01, 115.95; Purity by HPLC: 99.9% by area. UV/Vis in CH_3CN , λ_{max} nm ($\epsilon \text{ M}^{-1} \text{ cm}^{-1}$): 275 (84,300), 365 (19,300), 443 (16,600). ESI MS calcd for $\text{C}_{38}\text{H}_{24}\text{F}_2\text{N}_8\text{Ru}$: $[\text{M}^{2+}\cdot\text{PF}_6^-]^+$ 877.07, $[\text{M}]^{2+}$ 366.06, found 876.3 $[\text{M}^{2+}\cdot\text{PF}_6^-]^+$, 365.8 $[\text{M}]^{2+}$.

[Ru(bpy)₂dppz-11,12-Cl](PF₆)₂ (8).^{63,64}—The product was obtained in 81% yield (112 mg) as a red solid. ¹H NMR (CD₃CN, 400 MHz): δ 9.64 (d, *J* = 8.2 Hz, 2H), 8.53 (d, *J* = 8.3 Hz, 2H), 8.51 (d, *J* = 7.5 Hz, 2H), 8.17 (t, *J* = 5.5 Hz, 2H), 8.13–8.08 (m, 3H), 8.01 (t, *J* = 8.4 Hz, 2H), 7.96 (dd, *J* = 9.4, 2.7, 1H), 7.88 (ddd, 8.2, 5.4, 0.9 Hz, 2H), 7.85 (d, *J* = 5.1 Hz, 2H), 7.72 (d, *J* = 5.6 Hz, 2H), 7.47–7.43 (m, 2H), 7.25 (t, *J* = 6.7 Hz, 2H). ¹³C NMR (CD₃CN, 100 MHz): δ 164.02, 158.27, 158.06, 155.03, 154.80, 153.20, 153.06, 151.77, 151.44, 141.73, 141.40, 140.35, 139.07, 138.99, 134.71, 134.47, 133.53, 133.42, 131.84, 131.62, 128.72, 128.55, 125.42, 125.36, 124.73, 124.45, 113.66, 113.44; Purity by HPLC: 99.9% by area. UV/Vis in CH₃CN, λ_{max} nm (ε M⁻¹ cm⁻¹): 280 (92,300), 360 (18,100), 443 (17,500). ESI MS calcd for C₃₈H₂₄Cl₂N₈Ru: [M²⁺•PF₆⁻]⁺ 909.01, [M]²⁺ 382.03, found 908.8 [M²⁺•PF₆⁻]⁺, 382.6 [M]²⁺.

[Ru(bpy)₂dppz-11,12-Br](PF₆)₂ (9)—The product was obtained in 62% yield (94 mg) as a red solid. ¹H NMR (CD₃CN, 400 MHz): δ 9.59 (dd, *J* = 8.2, 1.2 Hz, 2H), 8.85 (s, 2H), 8.58 (d, *J* = 8.2 Hz, 2H), 8.55 (d, *J* = 8.2 Hz, 2H), 8.21 (dd, *J* = 5.1, 1.2 Hz, 2H), 8.14 (td, *J* = 8.2, 1.6 Hz, 2H), 8.05 (td, *J* = 7.4, 1.2 Hz, 2H), 7.91 (dd, *J* = 8.2, 5.5 Hz, 2H), 7.88–7.86 (m, 2H), 7.76–7.74 (m, 2H), 7.49 (ddd, *J* = 7.8, 5.7, 1.6 Hz, 2H), 7.29 (ddd, *J* = 7.2, 5.9, 1.2 Hz, 2H). ¹³C NMR (CD₃CN, 100 MHz): δ 158.25, 158.06, 155.28, 153.23, 153.05, 151.86, 142.77, 142.06, 139.12, 139.04, 134.65, 134.58, 131.48, 129.84, 128.73, 128.70, 128.60, 125.44, 125.39; Purity by HPLC: 95.4% by area. UV/Vis in CH₃CN, λ_{max} nm (ε M⁻¹ cm⁻¹): 285 (104,300), 385 (23,100), 440 (16,300). ESI MS calcd for C₃₈H₂₄Br₂N₈Ru: [M²⁺•PF₆⁻]⁺ 996.91, [M]²⁺ 425.98, found 997.6 [M²⁺•PF₆⁻]⁺, 426.3 [M]²⁺.

Counterion Exchange

All complexes were converted to Cl⁻ salts prior to testing by dissolving 5–20 mg in 1–2 mL methanol loading onto an Amberlite IRA-410 chloride ion exchange column. The compounds were eluted with methanol, and the solvent was removed under vacuum.

HPLC analysis for purity

The purity of each Ru(II) complex were analyzed as previously reported.⁴³

Luminescence and photorejection studies

Table S1 provides detailed information of the different protein, nucleoside and DNA sequences tested with each complex. Bovine serum albumin (BSA), deoxyadenosine (dA), deoxyguanosine (dG), thymidine (dT), and deoxycytosine (dC) were resuspended in buffer to give a 1 mM stock. Calf thymus DNA (CT DNA) and other DNA sequences was resuspended and sonicated as reported previously.⁶⁵ All biomolecules are stored long term at –20 °C. Luminescence studies of **1–9** were completed as previously reported.⁴³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

Bpy	2,2'-bipyridine
Dppz	dipyrido-[3,2- <i>a</i> :2',3'- <i>c</i>] phenazine
MLCT	metal-to-ligand charge transfer
CT DNA	calf-thymus DNA
BSA	bovine serum albumin
CD	circular dichroism

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Figure 1.

A library of Ru(II) dppz complexes was synthesized through a condensation reaction using a common Ru(II) building block. Unless otherwise designated, R = H.

Figure 2.

Experimental set up of the 384-well plate screening assay, where 4 compounds can be evaluated simultaneously under 32 different conditions in triplicate. Example data set for **3** shows the luminescence response in the presence of different biomolecules. (A) Buffer, (B) BSA, (C) nucleosides, (D) single strand DNA (Seq1–4), (E) duplex DNA (Seq5–11), (F) damaged duplex DNA (Seq12–21) (G) triplex DNA (Seq22–24), and (H) i-motif and G-quadruplex DNA (Seq25–33). Note: biomolecules are ordered as they appear in Table S1.

Figure 3.

Comparison of the fold change in luminescence as compared to duplex DNA. (A) Triplex DNA; Seq22 (T-A-T triplex, black, ●), Seq23 (5'-triplex, blue, ■), and Seq24 (3' triplex, red, ▲). (B) G-quadruplex DNA; Seq27 (intermolecular G-quadruplex, black, ●), Seq28 (*c-myc* G-quadruplex, blue, ■), Seq29 (telomeric antiparallel basket G quadruplex, red, ▲), and Seq30 (telomeric mixed hybrid G-quadruplex, green, ▼).

Figure 4.

Discriminating between G-rich triplex and G-quadruplex DNA with similar three-dimensional structures. Cartoon representation of the different triplex and G-quadruplex structures (A) Seq23 (5' triplex); (B) Seq24 (3' triplex); (C) Seq32 (Tel26 G quadruplex); (D) Seq31 (wtTel26 G-quadruplex). (E) Luminescence ratio when bound to the G-quadruplex versus its respective triplex (Seq32:Seq23 (blue), Seq31:Seq24 (green)). (F) Normalized ellipticity of the different DNA sequences (Seq23 (black), Seq32 (blue), Seq24 (red), and Seq31 (green)). (G) Ratio of the average response from Seq32 and Seq31 to Seq30. (H) Normalized ellipticity of the different mixed-hybrid G-quadruplex structures (Seq32 (blue), Seq31 (green), and Seq30 (orange)).

Figure 5.

Distinguishing different G-quadruplex structures: Seq27 (intermolecular G-quadruplex, dark blue), Seq28 (*c-myc* G-quadruplex, light blue), Seq29 (telomeric antiparallel basket G-quadruplex, dark red), Seq30 (telomeric mixed-hybrid G-quadruplex, orange).

Luminescence response in the presence of different G-quadruplex structures for the (A) F-substituted series; (B) Cl-substituted series; (C) Br-substituted series. Fold change (luminescence area in the presence of the different G-quadruplex structures in comparison to CT DNA) for the (D) F-substituted series; (E) Cl-substituted series; (F) Br-substituted series

Figure 6. Sensitivity to sequential G-quadruplexes. (A) Luminescence area of each complex in the presence of Seq30 (telomeric mixed-hybrid G-quadruplex, orange), and Seq33 (double mixed-hybrid G-quadruplex, purple); (B) Ratio of the Seq33:Seq30.

Figure 7.

Cartoon representation of different G-quadruplex structures. Colored blocks represent the anti configuration. (A) Seq27 (intermolecular G-quadruplex); (B) Seq28 (*c-myc* G-quadruplex); (C) Seq29 (telomeric antiparallel basket G-quadruplex); (D) Seq30 (telomeric mixed-hybrid G-quadruplex).