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[Ru(Me$_4$phen)$_2$dppz]$^{2+}$, a Light Switch for DNA Mismatches

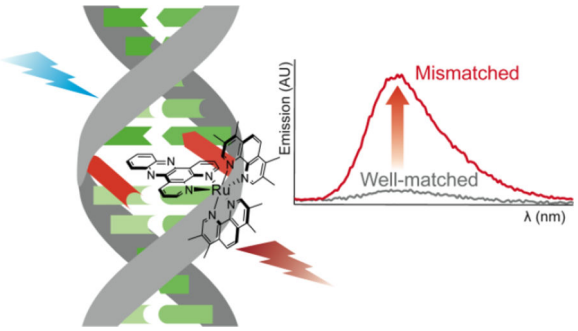
Adam N. Boynton, **Lionel Marcélis**, and **Jacqueline K. Barton***

Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125, United States

Abstract

[Ru(Me$_4$phen)$_2$dppz]$^{2+}$ serves as a luminescent "light switch" for single base mismatches in DNA. The preferential luminescence enhancement observed with mismatches results from two factors: (i) the complex possesses a 26-fold higher binding affinity towards the mismatch compared to well-matched base pairs, and (ii) the excited state emission lifetime of the ruthenium bound to the DNA mismatch is 160 ns versus 35 ns when bound to a matched site. Results indicate that the complex binds to the mismatch through a metalloinsertion binding mode. Cu(phen)$_2^{2+}$ quenching experiments show that the complex binds to the mismatch from the minor groove, characteristic of metalloinsertion. Additionally, the luminescence intensity of the complex with DNA containing single base mismatches correlates with the thermodynamic destabilization of the mismatch, also consistent with binding through metalloinsertion. This complex represents a potentially new early cancer diagnostic for detecting deficiencies in mismatch repair.

Graphical abstract



DNA mismatches arise as a result of errors during replication, and deficiencies in mismatch repair (MMR) machinery are implicated in several forms of cancer.[1–3] As such, the design of small molecules that target DNA mismatches holds promise for chemotherapeutic and diagnostic applications. A class of octahedral rhodium complexes, bearing sterically expansive planar ligands, bind DNA mismatches with high selectivity and exhibit preferential cytotoxicity towards MMR-deficient cancer cells.[4–6] These compounds bind to

Corresponding Author. jkbarton@caltech.edu.

ASSOCIATED CONTENT

Supporting Information

Experimental methods and supporting figures S1–S5. This material is available free of charge *via* the Internet at http://pubs.acs.org

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DNA through metalloinsertion, in which the bulky ligand inserts into the duplex at the thermodynamically destabilized mismatch site, displacing the mismatched bases into the DNA groove.[7,8] Luminescent reporters of mismatches may represent early diagnostics of carcinogenesis. Several groups have documented the use of organic small molecules, including Thioflavin T, cationic perylenediimides, and bisanthracene macrocycles, for fluorometric mismatch detection.[9–12]

In an effort to develop new selective, signal-on probes for DNA mismatches, we have focused our attention on derivatives of $[Ru(bpy)_2dppz)]^{2+}$ (dppz = dipyridophenazine), which serve as molecular "light switches" for duplex DNA.[13] While these complexes do luminesce in aprotic solvents, in aqueous solution, their luminescence is quenched due to hydrogen bonding interactions between solvent water molecules and the phenazine nitrogen atoms of the dppz ligand. However, upon intercalation into well-matched duplex DNA, these compounds luminesce brightly owing to protection of the dppz ligand from the aqueous environment.[13–16] $[Ru(bpy)_2dppz]^{2+}$ derivatives have seen utility as structural probes, cellular imaging agents, and in the development of new cytotoxic and photoactive small molecules.[17–25]

Interestingly, $[Ru(bpy)_2dppz]^{2+}$ exhibits a some-what brighter emission in the presence of a DNA mismatch relative to completely well-matched DNA.[26] A crystal structure of the complex bound to an oligonucleotide duplex containing a mismatch revealed that, analogously to rhodium metalloinsertors, the ruthenium complex binds at the mismatch site in the minor groove through metalloinsertion.[27] $[Ru(bpy)_2dppz]^{2+}$ is not mismatch-specific, however, since it readily binds to well-matched sites in the DNA duplex through intercalation. Ruthenium complexes bearing expansive inserting ligands, such as 5,6-chrysenequinone diimmine (chrysi), have been investigated, and while it was found that these compounds show mismatch specificity in binding, they are not luminescent at ambient temperature.[28] $[Ru(bpy)_2dppz]^{2+}$ derivatives in which the inserting dppz ligand was directly functionalized have also been examined, but an improved luminescence differential between mismatched and well-matched DNA compared to $[Ru(bpy)_2dppz]^{2+}$ was not achieved;[28] the functionalization of ancillary ligands has not been investigated in this context.

Here we sought to attain mismatch specificity through ancillary ligand modification of the $[Ru(phen)_2dppz]^{2+}$ scaffold using 3,4,7,8-tetramethyl-1,10-phenanthroline (Me_4phen, Figure 1). We rationalized that incorporation of methyl groups on the ancillary ligands would disfavor binding to well-matched sites as a result of steric clashing between the ancillary ligands and the DNA backbone. Moreover, bulkier ancillary ligands would both disfavor deep intercalation of dppz at a matched site and favor shielding of the phenazine nitrogen atoms with insertion at a mismatched site.[29]

$[Ru(Me_4phen)_2dppz]^{2+}$ was synthesized in two steps (see Supporting Information), and a racemic mixture of the chloride salt was used for all DNA experiments. As expected, the complex is not luminescent in aqueous solution upon excitation at 440 nm (MLCT transition).

We studied the steady-state luminescence response of the complex towards a well-matched 27-mer DNA duplex and the analogous DNA duplex containing a single CC mismatch (Figure 1). Indeed, the ruthenium complex acts as a DNA light switch. Excitation in the presence of either duplex yields emission spectra centered at 650–660 nm (Figure 1). Importantly, we observe appreciable luminescence with the 27-mer containing the single CC mismatch in comparison to the same 27-mer sequence lacking a mismatch. From DNA titrations of $[Ru(Me_4phen)_2dppz]^{2+}$ (Figure S1), we calculate relative binding affinities of $6.8 \times 10^4\ M^{-1}$ and $1.8 \times 10^6\ M^{-1}$ for well-matched and mismatched sites, respectively (Table 1). Given the 26-fold difference in binding affinities, we can conclude that the complex is quite selective for binding to the single base mismatch.

To determine whether the differential luminescence observed in the steady-state experiments is due not only to a higher binding affinity towards the mismatch but also to an increase in relative emissivity, we measured excited state emission lifetimes of $[Ru(Me_4phen)_2dppz]^{2+}$ with the well-matched and mismatched 27-mer (Table 1). In the presence of the well-matched sequence, a short emission lifetime equal to 35 ns is detected. However, with the mismatched duplex, the luminescence decays as a bi-exponential function with components equal to 33 ns and 160 ns. We attribute this additional longer lifetime component, 81% of the overall decay, to the population of excited complex bound to the mismatch. This longer-lived component is similar in luminescence lifetime of the complex in dry acetonitrile (Table 1). This similarity in excited state lifetime illustrates how effectively the inserted complex is protected from quenching within its mismatched binding site. Given the similarity in emission lifetimes between the short components of the well-matched and mismatched sequences and their % contributions, we assign the short component to ruthenium bound to well-matched sites, rather than enantiomeric differences.

We also investigated whether $[Ru(Me_4phen)_2dppz]^{2+}$ is capable of probing other types of DNA base mismatches using hairpin oligonucleotides (Figure 2) containing a variable base pair (XY). The emission intensity of the complex with the well-matched GC and AT sequences is compared to GG, AA, CT, TT, CA, and CC mismatches, as well as an abasic site (CR). The greatest emission enhancement occurs in the presence of the most thermodynamically destabilized mismatch, CC, followed by CA. We detect negligible enhancement with the GG mismatch as expected given its stability. Only a small enhancement is observed with the AA mismatch, which is generally more stable than CC, CA, and CT mismatches.[30,31] Figure 2 shows similar emission intensities for CT and TT mismatches, although we might anticipate a greater emission intensity for CT based on relative stabilities; we have previously noted that for $[Ru(bpy)_2dppz]^{2+}$, hydrogen bonding interactions between thymine and the dppz ligand at the mismatch may lead to partial quenching.[26] Note that some luminescence is evident with the fully well - matched hairpins. We attribute this luminescence to binding at the bulged hairpin site; metalloinsertion at bulged DNA sites has been observed.[32] We also examined the luminescence response towards an abasic site (CR), and we find that the enhancement is comparable to that with the CC mismatch, consistent with relative stabilities. Generally, then, the relative thermodynamic destabilization of the mismatch site correlates with the luminescence intensities seen in Figure 2. This dependence on the instability of the mismatch is consistent with metalloinsertion.[33]

Metalloinsertion by octahedral metal complexes occurs from the minor groove side of DNA. To probe whether $[Ru(Me_4phen)_2dppz]^{2+}$ does in fact bind at the mismatch from the minor groove, we tested $Cu(phen)_2^{2+}$ as a minor groove quencher (Figure 3).[27,34,35] With the DNA mismatch, as [Cu]/[Ru] increases, there is significant quenching of $[Ru(Me_4phen)_2dppz]^{2+}$ luminescence (Figure 3). Conversely, with well-matched DNA, there is little change in luminescence with increasing $Cu(phen)_2^{2+}$ concentration. These observations indicate ruthenium binding at the mismatch *via* the minor groove, consistent with metalloinsertion.[36] Moreover, the results suggest that binding to well-matched sites by the Me_4phen derivative occurs through the major groove.[37]

To help explain the differential luminescence observed between the mismatched and well-matched DNA samples, we explored models of the complex bound to well-matched and mismatched sites. Using the DNA coordinates from the crystal structure of Δ-$[Rh(bpy)_2(chrysi)]^{3+}$ bound by metalloinsertion to an AC mismatch,[7] we oriented Δ-$[Ru(Me_4phen)_2dppz]^{2+}$ into the mismatch site from the minor groove while minimizing steric clashes with the Me_4phen ancillary ligands and DNA (Figure 4). From this view, we can see that the dppz ligand is capable of deeply inserting into the mismatch site, allowing for significant protection from quenching by water. We also modeled major groove binding using the coordinates for intercalation by another rhodium complex.[38] For intercalation at a well-matched site, we consider two possible binding orientations:[15,29] (i) the dppz ligand intercalates in a “head-on” fashion (Figure S2), leaving both phenazine nitrogen atoms relatively well surrounded by the base stack; (ii) the dppz ligand binds “side-on”, achieving overlap with the base but with one of the phenazine nitrogen atoms being highly exposed to solvent quenching (Figure 4). Given the very short 35 ns lifetime observed for the complex with the well-matched duplex, we hypothesize that this side-on intercalation is the dominant binding mode when $[Ru(Me_4phen)_2dppz]^{2+}$ is bound to a well-matched site.[39]

By incorporating methyl groups onto the ancillary ligands of the $[Ru(phen)_2dppz]^{2+}$ scaffold, we have thus prepared a luminescent light switch that is highly selective in probing a DNA mismatch. This selectivity is the result of both a higher binding affinity towards mismatched DNA and a longer excited state emission lifetime when bound to a mismatch. This work demonstrates that ancillary ligand modification offers a new approach in the design of mismatch-specific transition metal complexes. Importantly, this complex represents a potential diagnostic probe for detecting early mismatch repair-deficient cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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36. As with other metalloinsertors, $[Ru(Me_4phen)_2dppz]^{2+}$ exhibits cell-selective cytotoxicity towards mismatch repair deficient cells (See SI).
37. Quenching studies with NaI also support binding to well-matched sites from the major groove (Figure S3).

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39. We also prepared $[Ru(5,5'\text{-dimethylbpy})_2dppz]^{2+}$ (Figure S4) This light switch exhibits a 7-fold luminescence differential between mismatched and well-matched sequences, an improvement over $[Ru(bpy)_2dppz]^{2+}$ but not as large as with the Me_4phen complex.

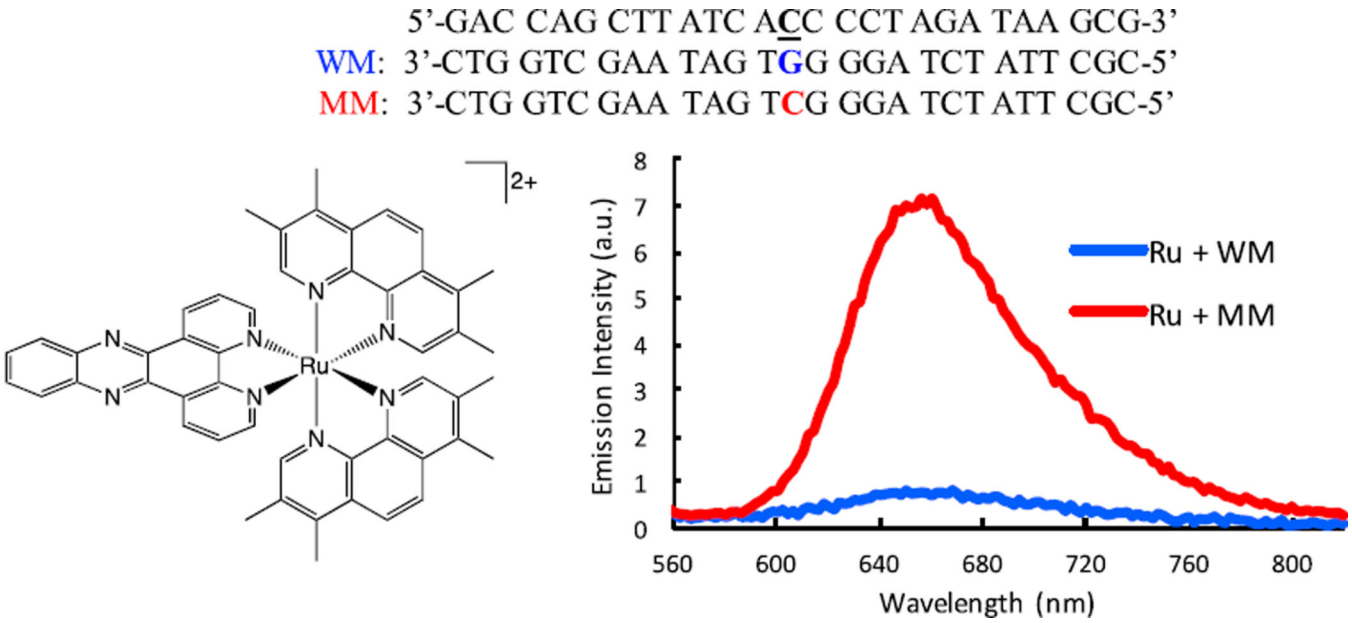

Figure 1.

(Top) DNA sequences used in this study. (Bottom left) Schematic of $[Ru(Me_4phen)_2dppz]^{2+}$. (Bottom right) Steady-state luminescence spectra of *rac*-$[Ru(Me_4phen)_2dppz]^{2+}$ with the well-matched (blue) duplex and with the duplex containing a single base pair CC mismatch (red). Samples were in 5 mM tris, 200 mM NaCl, pH 7.5. [Ru] = 2 μM, [DNA duplex] = 2 μM, λ_{ex} = 440 nm.

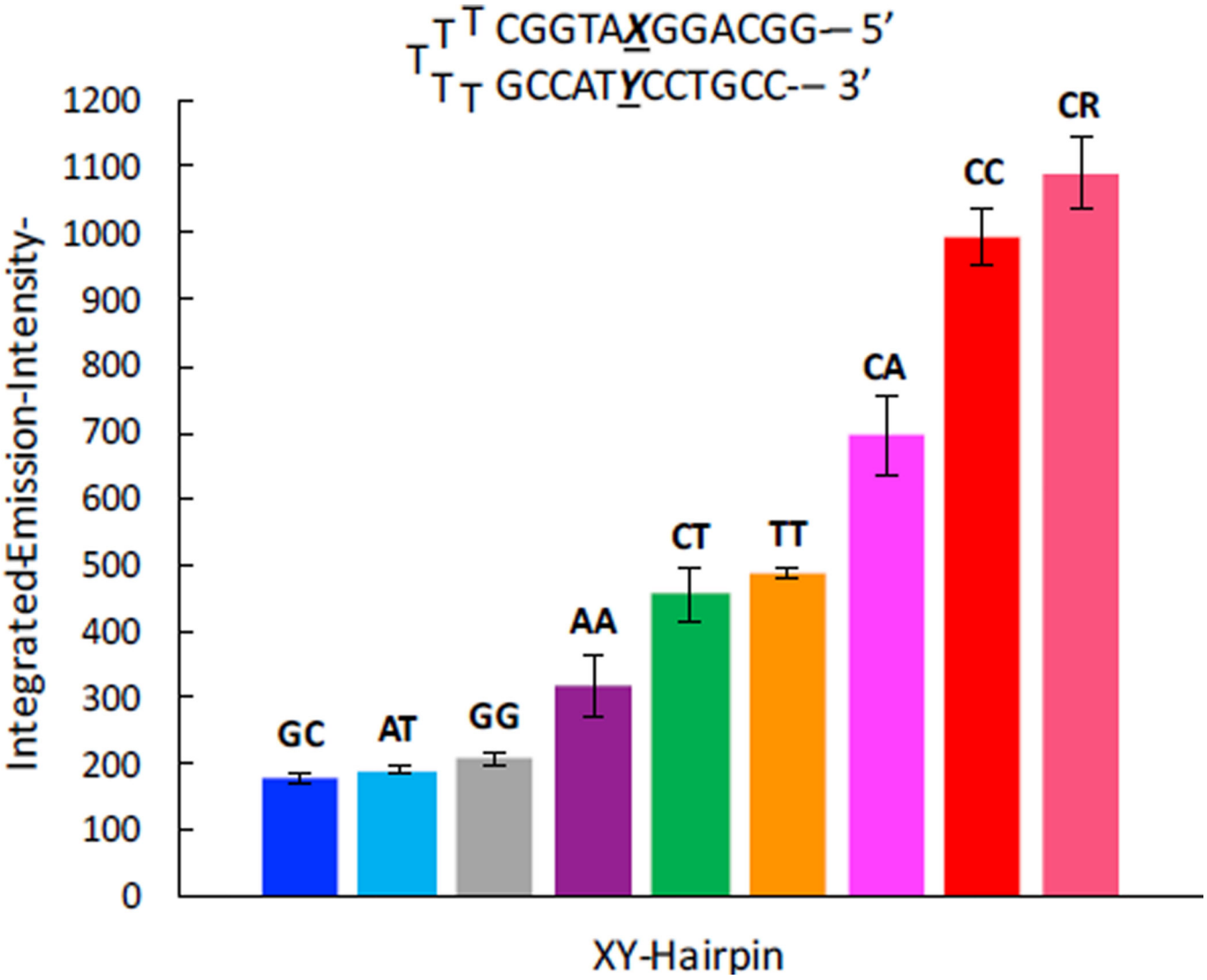

Figure 2.

Plot of integrated emission intensity of $[Ru(Me_4phen)_2dppz]^{2+}$ (2 μM) with DNA hairpins (2 μM) containing a variable XY base pair. "R" denotes a tetrahydrofuranyl abasic site. λ_{ex} = 440 nm. Samples prepared in 5 mM tris, 50 mM NaCl, pH 7.5. Error bars indicate standard deviations of three replicates.

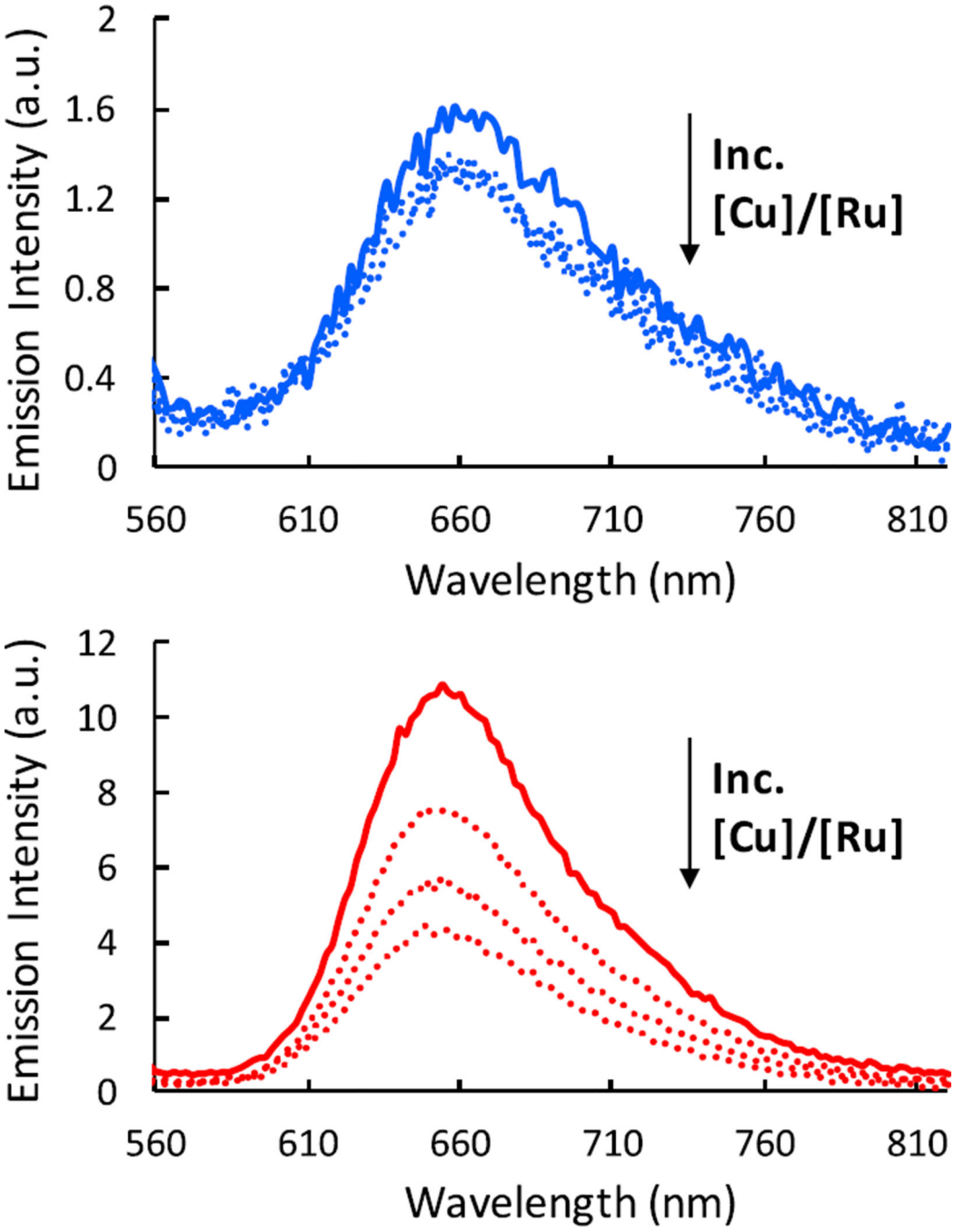

Figure 3.

Steady-state $Cu(phen)_2^{2+}$ quenching of $[Ru(Me_4phen)_2dppz]^{2+}$ (2 μM) bound to well-matched (top, blue) and mismatched (bottom, red) DNA (2 μM). Solid lines indicate no Cu present, and dotted lines, increasing concentrations of Cu such that [Cu]/[Ru] = 7, 40, and 100, respectively. λ_{ex} = 440 nm. Samples prepared in 5 mM tris, 50 mM NaCl, pH 7.5. The DNA sequences are as in Figure 1.

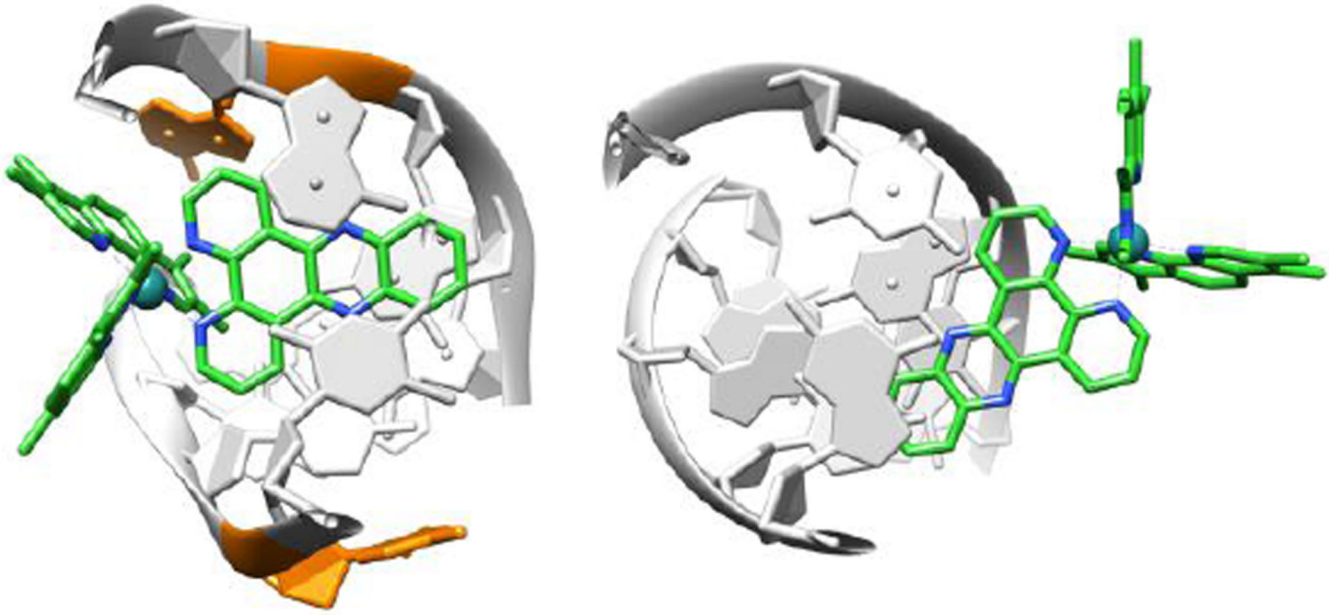

Figure 4.

Views down the helix axis of Δ-[Ru(Me_4phen)$_2$dppz]$^{2+}$ modeled into the crystal structures of DNA duplexes. The ruthenium complex is shown in green with nitrogen atoms in blue. Left: metalloinsertion at a mismatch site from the minor groove; the extruded mismatched bases are shown in orange. Right: side-on intercalation at a well-matched site from the major groove.

Table 1

DNA Binding Affinities of $[Ru(Me_4phen)_2dppz]^{2+}$ and Luminescence Life-times

	Well-matched DNA	**Mis-matched DNA**	**CH_3CN**
$K_a(M^{-1})$[a]	6.8×10^4	1.8×10^6	-
Emission Lifetime (ns)[b]	35	33 (19%) 160 (81%)	189[c]

[a] Titrations were performed with DNA sequences shown in Figure 1 in 5 mM tris, 200 mM NaCl, pH 7.5. [Ru] = 2 μM, λ_{ex} = 460 nm. The binding affinity is expressed per binding site, see SI.

[b] Samples containing 4 μM Ru and 4 μM DNA were prepared in 5 mM tris, 200 mM NaCl, pH 7.5 using DNA sequences shown in Figure 1. λ_{ex} = 440 nm, λ_{em} = 660 nm. Percentages reflect relative contributions of each lifetime to the overall decay.

[c] Obtained in degassed, anhydrous acetonitrile.