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A new type of DNA "light-switch": a dual photochemical sensor and metalating agent for duplex and G-quadruplex DNA†

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Ru(bpy)$_2$dppz, a well studied "light-switch" metal complex, transforms into a photochemical "light-switch" and DNA damaging agent by incorporating structural strain. This distorted compound is photoreactive and ejects a ligand upon binding duplex and G-quadruplex DNA, producing a reactive metal center that metalates the DNA.

Ruthenium complexes containing the dipyridylphenazine (dppz) ligand are important biological probes due to their sensitive "light-switch" behavior with DNA,[1] where they exhibit both high binding affinity and greatly increased emission in the presence of nucleic acids.[2] The Ru^{II} complexes are also emissive when surrounded by non-polar aprotic solvents, while exposure to protic solvents, especially water, quenches emission. A variety of compounds containing dppz and related ligands have been described,[3,4] and the photophysical process controlling the DNA-sensing properties have been explored, where multiple models have been proposed to explain this "light-switch" behavior.[3,5] Recently, studies have shown specific Ru^{II} dppz compounds display sensitivity to factors such as temperature,[6] DNA defects,[7] sequence, and ionic strength,[8] providing additional sensing capabilities.

Here we report a new type of DNA "light-switch": a ruthenium complex containing a dppz derivative that undergoes *photochemical* ligand substitution reactions in the presence of DNA. This is in marked contrast to the extensively described *photophysical* processes that have previously defined dppz complexes, and indeed most other biological probes. This unusual system exhibits selective photochemistry to generate a ligand-deficient and reactive metal center, along with a free coordinating ligand, in the presence of nucleic acids and organic solvents. Furthermore, the compound is sensitive to the DNA tertiary structure, displaying different reactivities in duplex and G-quadruplex DNA. This feature could provide the basis for the development of DNA structure-selective probes and effectors.

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† Electronic supplementary information (ESI) available: Experimental details, crystal structures, gel electrophoresis, photoejection UV-Vis and kinetic plots, chromatograms, and luminescent data. CCDC 962665 and 962666. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c3cc47269h

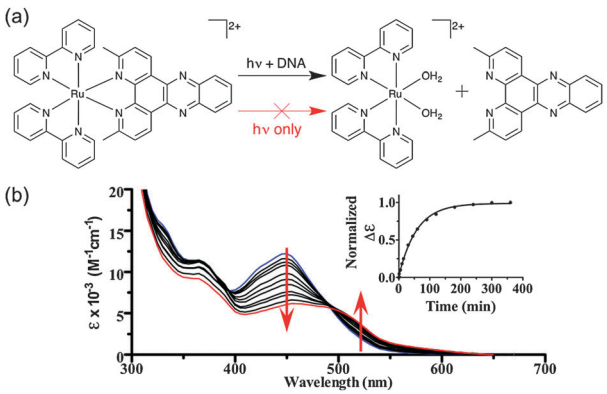

Fig. 1 (a) Scheme for the selective photoejection of dmdppz from **2** with $\lambda > 400$ nm light. (b) UV/Vis monitoring of photoejection in the presence of CT DNA (10 : 1 nucleotide: **2**). The kinetic fit for the photochemical reaction is shown in the inset.

We have previously developed strained ruthenium complexes that undergo phototriggered ligand ejection reactions in order to generate highly potent cytotoxic species that are only activated when exposed to visible or near-IR light.[9,10] This new light-responsive agent adds sensing functionality, giving a DNA-selective "photochemical light-switch". The ligand ejection proceeds through reactions of high-energy, ligand-dissociative metal centered (^{3}MC) excited states.[11] Direct excitation to the ^{3}MC state is forbidden, but intramolecular strain lowers the energy of the ^{3}MC state to allow for thermal population from lower energy metal-to-ligand charge transfer (MLCT) excited states, which are accessible with visible light.[12] Thus, to transform the light-switch Ru(bpy)$_2$dppz (**1**) into a probe that combines DNA sensing with ligand ejection photochemistry, as shown in Fig. 1, strain-inducing methyl groups were incorporated at the 3 and 6 positions of the dppz ligand (3,6-dimethyl dipyridylphenazine, dmdppz) to make complex **2**.

The crystal structures of racemic Ru(bpy)$_2$dppz (**1**) and Ru(bpy)$_2$-dmdppz (**2**) confirmed that addition of the methyl groups induced distortion about the metal center, as shown in Fig. 2; Fig. S15 and S16 and Tables S2 and S3 (ESI†). The Ru–N bonds are lengthened in the strained ligand, with an average of 2.10 Å for the Ru–N bonds in

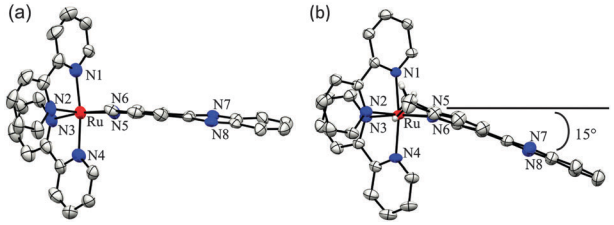

Fig. 2 Crystal structure of racemic (a) **1** and (b) **2** show the bend of the dmdppz ligand. Ellipsoids are drawn to 50% probability and hydrogen atoms are excluded for clarity. Note: the crystal structures contain two independent but structurally similar cations; only one is shown for clarity.

dmdppz, in contrast to 2.07 Å for dppz. The main distortion in compound **2** is a 15° bending of the dmdppz ligand from the normal plane of the octahedral complex, due to the clash of the methyl group with the auxiliary ligands. The distortion is limited to the misdirected[13] and extended metal–ligand bonds to the dmdppz ligand; the bpy ligands in either compound do not experience significant distortions.

Based on our previous work with analogous methylated, strained bipyridine or phenanthroline complexes,[9,14] one might expect complex **2** to undergo rapid photosubstitution reactions in water and other nucleophilic solvents. However, **2** is quite unreactive in water, exhibiting only very slow photodecomposition when exposed to visible light (λ > 400 nm; $t_{1/2}$ > 8 hours), hinting at a more discerning switch that could exploit triggers in concert with light. Indeed, the photoreactivity is increased by more than an order of magnitude in the presence of duplex DNA oligonucleotides and calf thymus (CT) DNA, with $t_{1/2}$ values of 31–41 minutes, as shown in Table 1. The photoejection process is characterized by both a red shift in the absorption spectra and decreased extinction coefficient. Ligand ejection is selective, as indicated by the presence of isosbestic points in the UV/Vis spectra taken as a function of irradiation time (Fig. 1). Only the dmdppz ligand is ejected, and the photoreaction leads to covalent metalation of the DNA (see Fig. S1 and S2, ESI†).

The distortion of compound **2** does not appear to significantly affect the DNA binding affinity, as similar K_b values of 1×10^7 and 3×10^7 M^{-1} were determined for racemic mixtures of **1** and **2** with CT DNA.[15] Intercalation of both complexes is also consistent with the reduced mobility of supercoiled plasmid DNA in agarose gels, and the modulation of the absorption spectra in the presence of DNA, characterized by hypochromism of both the π–π* of the dppz ligand and the MLCT transitions, with maximal effect at a ratio of 2 : 1 DNA base pairs to metal center (see ESI†).[16] Thus, the strained complex retains the key DNA-sensing capabilities of other Ru^{II} "light-switch" molecules with the added facet of photochemical reactivity.

Table 1 Photophysical and photochemical properties for **1** and **2** for various reaction conditions

Experimental conditions[a]	λ_{max} (nm) (**1**)	Half-life (minutes) (**2**)
H_2O	—	>480
D_2O	605 (weak)	140 ± 10
CH_2Cl_2	596	0.5 ± 0.05
DMF	638	25 ± 2
CT DNA	619	41 ± 2
15 mer oligonucleotide A	615	31 ± 0.5
G-Quadruplex	613	13.5 ± 1
BSA	—	146 ± 8

[a] See ESI for experimental details, UV/Vis plots and kinetic profiles.

Alternative tertiary structures of DNA are intriguing targets for small molecule probes and therapeutics as they provide potential for greater selectivity in gene and cellular regulation than double stranded motifs. G-quadruplex structures are stacked tetrads of guanine bases found in both telomere regions of chromosomes and promoter regions of specific oncogenes,[17] making them medically significant targets for cancer research and therapies.[18] It has been shown that Ru^{II} complexes containing the dppz ligand bind to G-quadruplexes and exhibit enhanced emission,[19] so **2** was tested as a photochemical probe for the telomeric G-quadruplex structure using the sequence [AGGG(TTAGGG)$_3$]. As anticipated, upon binding the quadruplex, the photoejection rate for **2** increased 3-fold compared to standard double helix CT DNA, giving a $t_{1/2}$ of 13.5 minutes.[20] This greatly enhanced reactivity offers promise for **2** in potential applications in sensing and perturbing G-quadruplexes,[21] and suggests it may show other sequence or structure discrimination abilities.

The selectivity of the probe for DNA was compared to protein by testing the ejection with bovine serum albumin (BSA), a model non-selective small molecule binding protein.[22] In contrast to the nucleic acids, there was no observable emission for **1** in the presence of BSA, and **2** appeared to photodecompose, with no isosbestic points in the absorption spectra (Fig. S11, ESI†) and a $t_{1/2}$ of 146 minutes. The poor reactivity with a hydrophobic protein is similar to the behaviour in water, and highlights the selectivity of the photochemistry for the environment provided to the probe by intercalation in DNA.

Overall, the photochemical behaviour of **2** parallels the photophysical characteristics of unstrained Ru^{II} complexes containing the dppz ligand. For example, the emission intensity of **1** and other dppz complexes increases significantly in nonpolar, aprotic solvents such as dichloromethane;[23] similarly, the photoejection rate of **2** increases markedly as well ($t_{1/2}$ > 8 hours in water, $t_{1/2}$ = 0.5 min in CH_2Cl_2, providing >1000-fold increase in ejection rate). As CH_2Cl_2 is a poor coordinating solvent, ligand ejection is likely followed by coordination to the Cl^- counterion, producing a red-shift in the absorption spectra (Fig. S5–S7, ESI†). The luminescence of Ru^{II} dppz complexes is greater in D_2O than H_2O;[24] the ejection of **2** also shows a similar isotope effect of >3.4.[25] These results are consistent with the interpretation of quenching of the excited state photoejection process through vibrational deactivation processes mediated *via* H-bonding.

The current model that describes the enhanced emission of the Ru^{II} dppz complex when bound to DNA or dissolved in non-polar solvents is based upon a competition between a lower energy "dark" state that arises from the Ru^{II} MLCT to the phenazine portion of the dppz ligand and a higher energy "bright" state that is the result of an MLCT to the bipyridine portion of the dppz ligand. The relative energies of these two states are sensitive to the environment about the complex.[26] Polar, protic environments stabilize the "dark" state, lowering its energy and preventing the thermal population of the higher energy "bright" state, thereby quenching emission.[3,26] In contrast, in DNA or aprotic solvents the "dark" state is close enough in energy to the "bright" state to allow for its thermal population, resulting in enhanced emission.

The photochemical behaviour of compound **2** in DNA and select solvents is well explained by this model, augmented by a tunable and energetically accessible ^{3}MC state. A proposed Jablonski diagram is

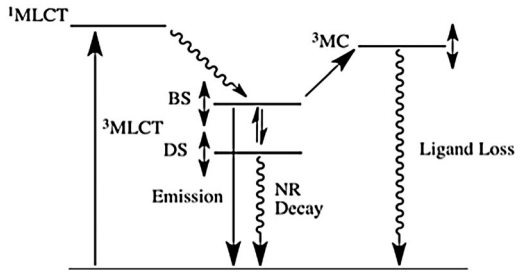

Fig. 3 Simplified Jablonski diagram for **2** showing the response of the different excited states to environment and strain. BS = bright state; DS = dark state; NR decay = nonradiative decay.

shown in Fig. 3 illustrating the interplay between these states. The "bright" and "dark" states are in thermal equilibrium as a function of the environment, and the dissociative ^{3}MC state can be accessed only from the "bright" state. Population of the "dark" state is enhanced in solvents such as water, and results in alternative non-radiative decay pathways that reduce the yield of photoejection. A correlation can also be drawn between the energy of the "bright" state (reflected by the emission wavelength of the unstrained complex **1**; Table 1) and the ejection rate of the strained system **2**. This is consistent with enhanced population of the ^{3}MC state from the "bright" state when the "bright" state is elevated in energy. Studies in non-aqueous solvents provide additional support for this explanation. Two aprotic solvents with similar $E_T(30)$ values were chosen to compare emission and photoejection properties; the $E_T(30)$ scale provides an empirical measure of the microscopic polarity of the solvent.[27] The emission quantum yields of **1** are similar in DMF and CH_2Cl_2, (DMF $E_T(30)$ = 43.8; Φ_{em} = 0.011; CH_2Cl_2 $E_T(30)$ = 41.1; Φ_{em} = 0.012).[23] However, a 50-fold difference in $t_{1/2}$ values was found for **2** (see Table 1) in CH_2Cl_2 and DMF. Emission in the slightly more polar DMF is red-shifted, with λ_{max} = 638 nm, reflecting a stabilization of the "bright" state, and thus reducing population of the ligand dissociative ^{3}MC state. In contrast, the emission in CH_2Cl_2 is characterized by λ_{max} = 596 nm, and much more facile ligand loss. This data suggests that the complex will undergo ligand ejection photochemistry most efficiently under conditions that destabilize polar charge transfer states and reduce population of the "dark" state MLCT and it associated non-radiative decay pathways.

To the best of our knowledge, this strained Ru^{II} dmdppz compound is the first metal complex shown to act as a dual photochemical DNA sensor and metalating agent. For this molecule, strain is necessary but not sufficient to induce photoejection, as environmental factors affect other nonradiative decay processes. Additional studies of the sensitivity of the probe to nucleic acid sequence and structure are currently underway, along with investigations of other structural and environmental features that may regulate the photoreactivity of the complex.

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