DNA Intercalators

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Tuning the Excited State of Water-Soluble Ir^{III}-Based DNA Intercalators that are Isostructural with $[Ru^{II}(NN)_2(dppz)]$ Light-Switch Complexes**

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Abstract: *The synthesis of two new Ir^{III} complexes which are effectively isostructural with well-established $[Ru(NN)_2(dppz)]^{2+}$ systems is reported (dppz = dipyridophenazine; NN = 2,2′-bipyridyl, or 1,10-phenanthroline). One of these Ir^{III} complexes is tricationic and has a conventional N_6 coordination sphere. The second dicationic complex has a N_5C coordination sphere, incorporating a cyclometalated analogue of the dppz ligand. Both complexes show good water solubility. Experimental and computational studies show that the photoexcited states of the two complexes are very different from each other and also differ from their Ru^{II} analogues. Both of the complexes bind to duplex DNA with affinities that are two orders of magnitude higher than previously reported Ir(dppz)-based systems and are comparable with Ru^{II}(dppz) analogues.*

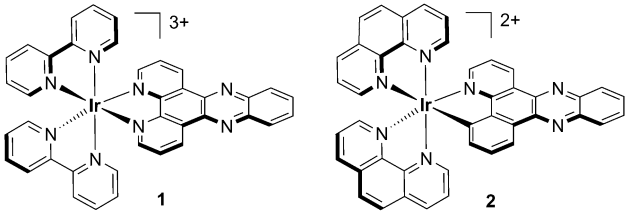
DNA has a vital role in life; as outlined in the "central dogma of molecular biology" it stores and transmits the genetic blueprints for structure and function in all living organisms. For this reason, molecules that target DNA have been intensively researched.

In this context, and inspired by the serendipitous discovery and subsequent clinical success of the potent anticancer agent cisplatin,[1,2] research into metal complexes that interact with DNA has burgeoned. More recently this work has been extended to yield an array of transition-metal-based nucleic acid probes as, because of an attractive combination of well-defined coordination geometries and substitution chemistry as well as distinctive electrochemical and photophysical properties, they are almost perfect candidates for such a role.[3–7]

Luminescent and photoreactive d^6 metal-ion-based complexes that intercalate into DNA have been particularly well-studied,[8–11] leading, inter alia, to the now well-characterized DNA light-switch effect exemplified by $[Ru(NN)_2(dppz)]^{2+}$ (where NN = 2,2′-bipyridyl (bipy), or 1,10-phenanthroline (phen), and dppz = dipyridophenazine).[12]

Concurrently, the coordination chemistry of another d^6 metal ion, Ir^{III}, has been rapidly developing. Polypyridyl Ir^{III} complexes are finding a range of applications, largely because their photoexcited states are much more tunable than their Ru^{II}-based analogues.[13–15] However, whilst such systems have been investigated as therapeutics[16,17] and as cell probes,[18] their use in these applications is often restricted as because of their relatively low charge, cyclometalated Ir^{III} complexes display poor inherent water solubility and DNA binding affinities. For example, several Ir^{III}(dppz) systems incorporating cyclometalated ancillary ligands have been previously reported, but these complexes display relatively low DNA binding affinities ($\approx 10^4\,M^{-1}$) compared to their Ru^{II}(dppz) analogues ($> 10^6\,M^{-1}$).[19,20]

As part of a program to develop metal-complex-based bioprobes with targeted binding properties and attractive photophysical/imaging properties, we set out to synthesize water-soluble, Ir^{III}-based metallointercalators that are isostructural analogues of the parent $[Ru(phen)_2(dppz)]^{2+}$ system. By adapting previously reported synthetic methods,[21,22] this has led to the preparation of the tricationic complex **1** (Scheme 1). We also investigated coordination of



Scheme 1. Structures of complexes **1** and **2** prepared in this study.

appropriate Ir^{III} moieties to the potentially cyclometalating dppz analogue benzopyridophenazine (bppz). Surprisingly, although this ligand has been reported before,[23] this is the first time its use in the construction of a DNA binding system has been investigated. In fact, to our knowledge this study provides the first example of its use in coordination chemistry.

While attempts to synthesize the dicationic analogue of **1** using bppz were unsuccessful, the closely related complex **2**, incorporating the $Ir^{III}(phen)_2$ moiety, was isolated in reasonable yields. The complexes were synthesized as hexafluoro-

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phosphate salts and then converted into chlorides by counterion metathesis; in this form both complexes were highly water soluble.

A comparison of the optical properties of **1** and **2** shows the effects of cyclometalation on the Ir^{III} center. The UV/Vis absorption spectra of both complexes (Figure S1 in the Supporting Information) show high-energy bands below $\lambda = 300$ nm that are assigned to ligand-centered $\pi \rightarrow \pi^*$ transitions. While complex **1** shows a "double-humped" structured band centered at approximately $\lambda = 360$ nm that is characteristic of a coordinated dppz ligand, complex **2** displays a broad, featureless absorption shoulder centered at $\lambda = 350$ nm that extends out beyond $\lambda = 400$ nm. Differences in the emission properties of the two complexes are more striking.

Unlike their Ru(dppz) analogues, both complexes are emissive in water (Figure 1). Photoexcitation of complex **1** results in an emission band with clear vibronic structure with

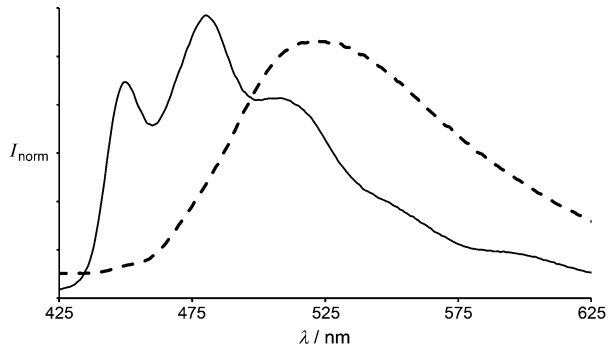

Figure 1. Normalized emission spectra of complexes **1** (solid line) and **2** (dashed line) in water.

a maximum emission centered at $\lambda = 479$ nm. In contrast, excitation of **2** results in a broad featureless emission centered at $\lambda = 522$ nm. These observations are in line with previous studies demonstrating that the energy and nature of emissive states in polypyridyl Ir^{III} complexes are modulated through coordination to cyclometalated ligands, and suggest that the excited state of **2** has a much greater MCLT (metal-to-ligand charge transfer) character than that of **1**.

To investigate these emission properties in more detail, DFT calculations were performed on both the S_0 state and the T_1 state of both **1** and **2** as well as on the bipy equivalent of **2** (**2′**).

Structurally, **1** and **2** are very similar as are **1** and **2′**. Indeed, **1** is also very similar to its Ru^{II} analogue (Tanimoto coefficient 0.997; see the Supporting Information for overlays). Interestingly, the frontier orbitals of **1** and **2** (particularly the virtual orbitals) are different (see the Supporting Information). Whereas the highest occupied molecular orbital (HOMO) of **1** is solely located on the dppz ligand, the HOMO of **2** has a significant contribution from the Ir^{III} center. Consistent with the experimental data, the calculated UV/Vis absorption spectrum of **1** and **2** (Figure S4) also shows that the absorption spectrum of **2** extends further into the lower energy region of the spectrum. Furthermore, whilst the two triplet states are structurally similar, they are

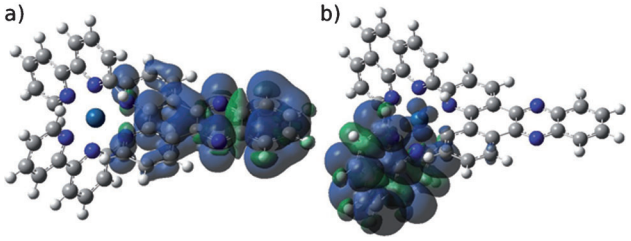

Figure 2. Spin-density plots for the T_1 state of complexes **1** (a) and **2** (b). Atom sphere colors: C = gray; H = white; N = dark blue; Ir = lighter blue.

clearly electronically different. As is shown in Figure 2, the spin density for the T_1 state of **1** is largely concentrated on the dppz unit. In contrast, for the T_1 state of **2** the spin density is located on the metal center and one of the phen units. Calculations on **2′** confirm that the change in excited state is a consequence of the cyclometalation, as for **2′** the spin density is also localized away from the dppz ligand. Calculation of the complete emission spectrum of both **2** and **1** was not possible with our current resources, but the 0–0 transition for **1** was calculated to be at $\lambda = 596.6$ nm, whereas the 0–0 emission for **2** is calculated at $\lambda = 475.3$ nm. For **1**, the calculated transition appears to be in reasonable agreement with the experimental data, whereas for **2** the calculated transition lies at higher energy than the experimental value.

Given that the two complexes are cationic and incorporate ligands with extended aromatic surfaces, the interaction of **1** and **2** with DNA was then investigated. It is well established that many complexes containing the Ru^{II}(dppz) moiety produce increases in relative viscosity on progressive addition to aqueous solutions of DNA[24] and this response is one of the clearest general diagnostics for an intercalative interaction.[25] Consequently, the effect of **1** and **2** on the viscosity of solutions of calf-thymus DNA (CT-DNA) was investigated.

As shown in Figure 3, both complexes induce significant positive viscosity changes that are indicative of intercalative binding. Interestingly, both complexes also initially induce a negative change in relative viscosity suggesting that, at low complex loading, non-intercalative interactions are occurring, a phenomenon that has been suggested before for Ru^{II}(dppz)-based systems.[26] However, it is also clear that complex **1** causes larger changes than **2**.

It seems likely that this effect may be due to the difference in charge between the two systems; the electrostatic contribution to association with the polyanionic backbone of DNA for the tricationic complex **1** should be higher than that of dicationic **2**, thus bringing about a closer association, although the influence of the different ancillary ligands may also be a factor. Having established that **1** and **2** do interact with DNA, their binding properties were further parameterized through luminescent titrations.

In stark contrast to their isostructural Ru^{II}(dppz) analogues, addition of CT-DNA to aqueous solutions of **1** or **2** results in a substantial decrease in steady-state luminescence (Figure 4). Although both complexes display a similar 5 nm blue shift in luminescence, the DNA-induced emission

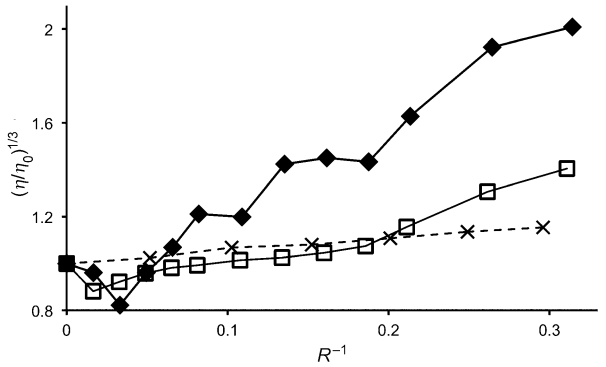

Figure 3. Plots of relative viscosity $(\eta/\eta)^{1/3}$ of CT-DNA versus R^{-1} (R = [DNA]/[ligand]) in buffered aqueous solutions on addition of complexes **1** (◆) and **2** (□) compared to the established intercalator ethidium bromide under the same conditions (×). The connecting lines are not a model fit, but an aid for visualization of data.

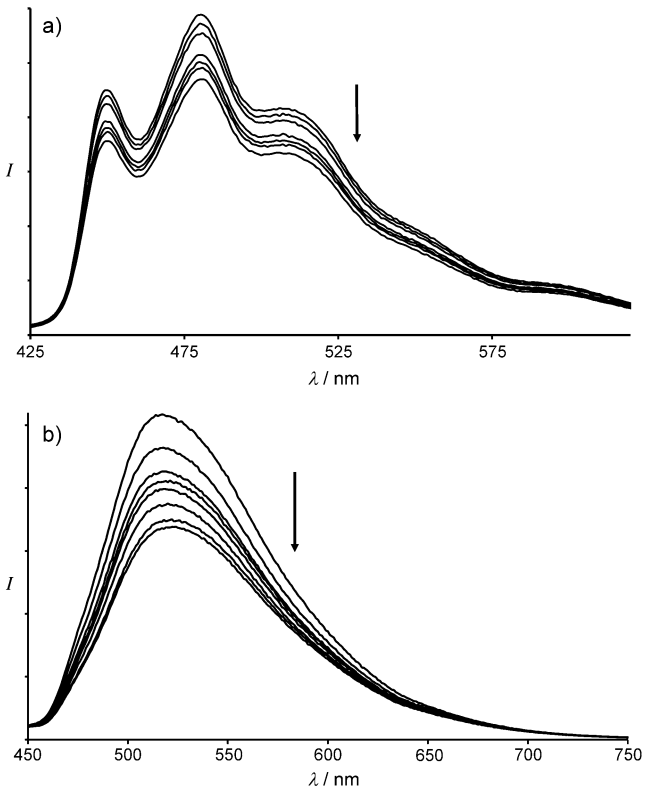

Figure 4. Changes in the emission spectra of aqueous solutions of complexes **1** (a) and **2** (b) with successive additions of CT-DNA.

decrease is much larger for complex **2** (>35%) compared to complex **1** (≈19%).Fits of this data to the commonly employed McGhee–von Hippel model (MVH) for non-cooperative binding[27] yields the binding parameter estimates summarized in Table 1. Strikingly, these data reveal that even though complex **2** has a lower cationic charge than **1** (within experimental error), both complexes have almost identical binding affinities. Another significant observation is that, unlike the previously Ir^{III}(dppz) systems, these affinities are highly comparable to the high affinities reported for their isostructural Ru^{II}-based analogues.

Table 1: CT-DNA binding parameters for complexes **1** and **2**.[a]

Complex	K_b [M^{-1}]	N (bp)
1	1.8×10^6	2.0
2	2.7×10^6	2.4

[a] Parameters obtained from MVH fits to the luminescence-based titration data. K_b = DNA binding affinity; N = site size.

These results are consistent with the interplay of two contributing factors to DNA binding. The decreased charge of **2** relative to complex **1** will decrease any electrostatic interactions of the complex with DNA. However, previous studies have shown that electrostatics do not provide a major contribution to the thermodynamics of the intercalative interaction, which is largely driven by hydrophobic[28,29] and electronic[30] effects, and it seems these contributions are more important in the M(dppz) system and its cyclometalated analogue. The DFT calculations explain why **1** and **2** do not display DNA light-switch effects. The excited state of **1** is best described as an intraligand state located on the dppz unit and previous studies have shown that such excited states are emissive in water and can be reduced on DNA binding.[31–34] In contrast, the excited state of **2** is more consistent with a high-energy MLCT transition involving a non-intercalative phen unit, which will not be quenched by water but can be redox quenched on interaction with DNA.

This study is the first to explore the DNA-binding properties of Ir^{III}-based isostructural analogues of the $[Ru(NN)_2(dppz)]^{2+}$ DNA light-switch systems. Surprisingly, it is also the first to investigate the DNA binding properties of a cyclometalated system based on the bppz ligand. This work shows that although the binding properties of these complexes are comparable to the parent Ru^{II} systems, the emission characteristics can be readily tuned. The decrease in intensity of the high-energy luminescence of both **1** and **2** on addition of DNA is particularly striking and is suggestive of redox quenching by nucleobase sites in DNA. This possibility is being explored and will form the basis of future reports. Given the well-established tunable nature of the excited states of polypyridyl Ir^{III} complexes, the potential of these systems and their derivatives for a range of applications, including as sensitizers for photodynamic therapy, is clearly apparent.

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