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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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((Dedication----optional))

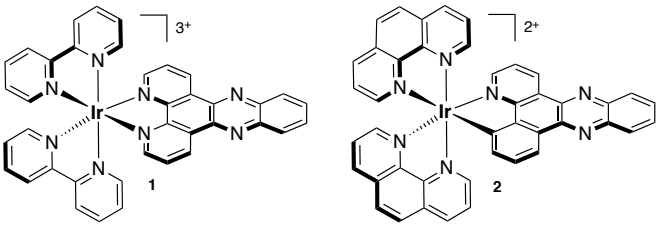
***Abstract**: The synthesis of two new Ir^{III} complexes that are effectively isostructural with much-studied $[Ru(NN)_2(dppz)]^{2+}$ systems are reported. One of these complexes is tricationic and has a conventional N_6 coordination sphere. The second dicationic complex has a N_5C coordination sphere, as it incorporates a cyclometallated analogue of dppz. Both complexes show good water solubility. Experimental and computational studies show that the photoexcited states of these two new complexes are very different from each other and also differs from their ruthenium(II) analogues. Both 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 the structure and function in all living organisms. For this reason molecules that target DNA are much researched.

In this context - inspired by the serendipitous discovery and subsequent clinical success of the potent anti-cancer 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, due to 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 photo-reactive d^6-metal-ion-based complexes that intercalate into DNA have been particularly studied;[8-11] *inter alia*, this work has led the now well-characterized DNA light switch effect exemplified by $[Ru(NN)_2(dppz)]^{2+}$ (where N-N = 2,2'-bipyridyl, or 1,10-phenanthroline, phen, and dppz = dipyridophenazine)[12]

Concurrently, the coordination chemistry of another d^6 metal ion 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 cell probes,[18] the use in these applications is often restricted as - due to 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 cyclometallated ancillary ligands have been previously reported, but these complexes display relatively low DNA binding affinities (~10^4 M^{-1}) compared to their Ru^{II}(dppz) analogues (>10^6 M^{-1}).[19,20]



Scheme 1 – Structures discussed in this study

As part of a program to develop metal complex-based bio-probes 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 tricationic complex **1**, Scheme 1. We also investigated coordination of 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, as far as we are aware, this study provides the first example of its use in coordination chemistry.

Whilst attempted syntheses of the dicationic analogue of **1** with bppz were not successful, closely related complex **2** - which incorporates the $Ir^{III}(phen)_2$ moiety - was isolated in reasonable yields. The complexes were synthesized as hexafluorophosphate salts and then converted to 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} centre. Both their absorption spectra – See SI Figure 1 – show high energy bands below 300 nm that are assigned to ligand-centred $\pi\rightarrow\pi^*$ transition, but whereas complex **1** shows a double humped structured band centred around ~360 nm that is characteristic of coordinated dppz, complex **2** displays a broad, unfeatured shoulder centred at 350 nm that extends out beyond 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. Photo-excitation of complex **1** results in a clearly vibronically structured emission displaying a maximum centred at 479 nm. In contrast, excitation of **2** results in a broad featureless

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emission centred at 522 nm (see Figure 1). 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 character than that of **1**. To investigate this 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'**).

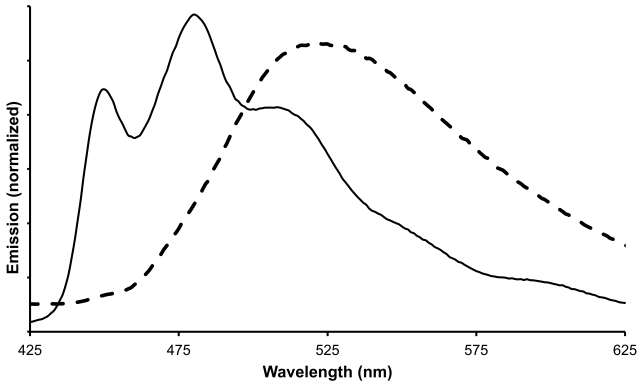

Figure 1. Normalized emission of complexes **1** (continuous line) and **2** (dashed bold line) in water.

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). For overlays, see SI. Interestingly, the frontier orbitals of **1** and **2** (particularly the virtual orbitals) are different (see SI). Whereas the HOMO of **1** is solely located on dppz, the HOMO of **2** has a significant contribution from the Ir^{III} centre. Consistent with the experimental data, the calculated absorption spectrum of **1** and **2** (See Figure S4) also shows that the absorption spectrum of **2** extends further into the red. Furthermore, whilst two triplet states are structurally similar, they are 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 and one of the phen-units. Calculations on **2'** confirm that the change in excited state is a consequence of the cyclometallation, since for **2'** the spin density is also localized away from dppz. 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** occurs at 596.6 nm, whereas the 0-0 emission for **2** is calculated to be at 475.3 nm. The former appears to be in reasonable agreement with the experimental data, whereas the latter lies to the blue of the experimental wavelength.

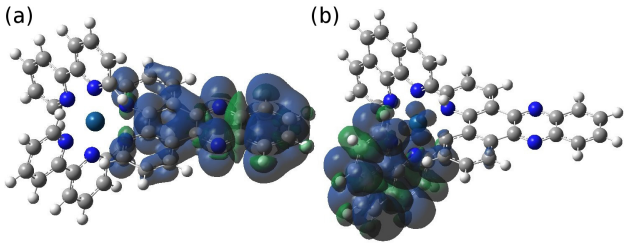

Figure 2. Spin density plots for the T_1 state of complexes **1** (a) and **2** (b).

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 CT-DNA solutions was investigated

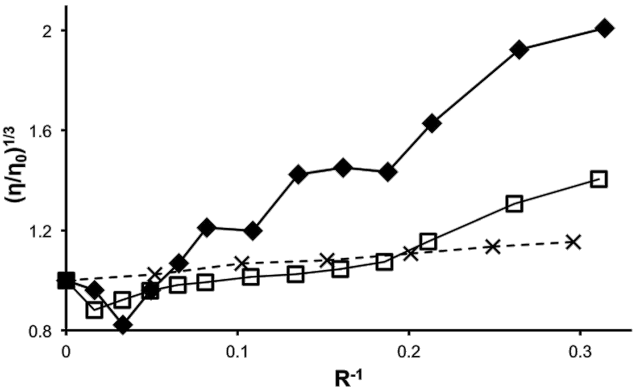

Figure 3. Relative viscosity changes in buffered aqueous solutions of CT-DNA on addition of complexes **1** (◆) and **2** (□) compared to the known intercalator ethidium bromide in the same conditions (×). The connecting lines are not a model fit, but an aid for visualization of data.

As illustrated by Figure 3, both complexes do 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 maybe 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 decrease is much larger for complex **2** (>35%) compared to complex **1** (~19%) .Fits of this data to the commonly employed McGhee-von Hippel, MVH, model for non-cooperative binding[27] yields the binding parameter estimates summarised in Table 1. Strikingly, these data reveal that - even though complex **2** has a lower cationic charge than **1** - within experimental error, both complexes possess almost identical binding affinities. Another significant observation is that, unlike the previously Ir^{III}(dppz) systems, these affinities highly comparable to the high-affinites reported for their isostructural Ru^{II}-based analogues.

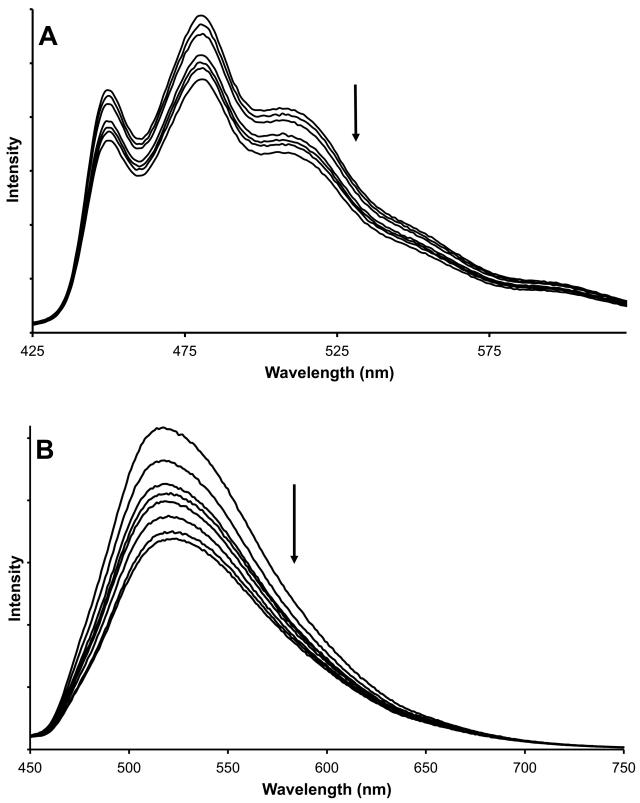

Figure 4. Changes in the emission of aqueous solutions of 1 (**A**) and 2 (**B**) on progressive addition of CT-DNA.)

Table 1. CT-DNA binding parameters for complex **1** and **2** obtained from MVH fits to the luminescence-based titrations.

Complex	Kb (M^{-1})	N (bp)
1	1.8 x 10^6	2.0
2	2.7 x 10^6	2.4

The observations are consistent with the interplay of two contributions to DNA binding. The decreased charge of **2** relative to complex **1** will reduce any electrostatic interactions 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 are reduced on DNA binding. [31-34] Whilst the excited state of **2** is more consistent with a high-energy MLCT 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. It illustrates that although the binding properties of these complexes are comparable to the parent Ru^{II} systems, the emission characteristics can be readily tuned. As outlined above, the reduction 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 tuneable nature of polypyridyl Ir^{III} complexes' excited states, the potential of these systems and their derivatives for a range of applications - including as sensitizers for photodynamic therapy – is clearly apparent.

Experimental Section

See Supporting Information for details of syntheses, computational procedures, UV-Visible spectra, and computational analyses as well as additional references.

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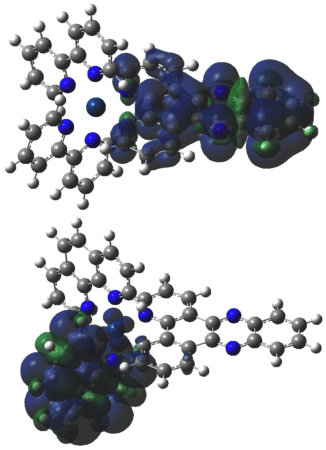

Two new complexes containing $Ir^{III}(NN)_2$ moieties coordinated to the well-known DNA intercalating ligand dppz and its cyclometalating analogue, benzopyridophenazine are reported. Experimental and computational studies show that both systems have very different excited states and both complexes bind to DNA with high affinities, that are comparable to their isostructural Ru^{II} analogues.