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Communication

Using ancillary ligands to tune the DNA binding properties of self-assembled luminescent metallomacrocycles

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The optical response, binding parameters, and duplex DNA binding mode of water-soluble kinetically inert tetranuclear metallomacrocycle can all be controlled by judicious selection of ancillary ligands.

In recent years metal-directed self-assembly has emerged as a versatile route towards the construction of complex molecular architectures.[1-4] In particular, work in this area has produced hosts and sensors for a wide range of molecular guests.[5-7] Whilst octahedral metal ion-based triple helicates that interact with biomolecules have been much investigated,[8-11] surprisingly little work on self-assembled cages and macrocycles that recognize
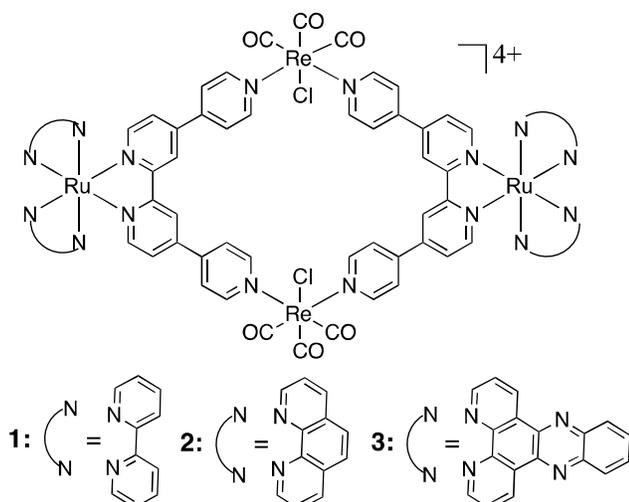

Fig. 1 self-assembled metallomacrocycles used in this study.

biomolecules such as DNA, in biologically relevant conditions has been reported.

The Therrien group has previously reported on a number of cages constructed from arene ruthenium fragments that can be used for *in cellulo* delivery of molecular packages.[12] In collaboration with the Vilar group they have shown that related systems can bind to both duplex and quadruplex DNA.[13] More recently, the Chi and Stang groups have also shown that heterometallic macrocycles comprised of ferrocene fragments linked by Pt^{II}-centres also bind to DNA – albeit with low affinity (~10^3 M^1) – resulting in DNA unwinding.[14] However, in these cases, the exact binding mode with duplex DNA has not been delineated. In contrast, a detailed study has shown that the platinum-based supramolecular square first

reported by Fujita preferentially binds to quadruplex over duplex DNA through a putative end-stacking interaction.[15]

In the first study of this kind on an emissive system, we reported on a large, kinetically-inert, tetranuclear metallomacrocycle, **1**, based on the 2,2':4,4'':4',4'''-quaterpyridyl, qtpy, bridging ligand that binds duplex DNA with high affinity through a non-intercalative external mode that resembles binding by DNA recognition proteins such as the TATA box binding protein, as it produces large-scale DNA bending. Furthermore, the distinctive Ru(II)→qtpy ^{3}MLCT luminescence of the macrocycle decreases when it binds to duplex DNA.[16]

In this study we employ the previously reported macrocycles[17] **2** and **3**, Fig. 1, to explore whether changes in the ancillary ligand set around the central macrocyclic core of **1** affects its DNA binding properties. Like **1**, macrocycles **2** and **3** also bind anions and aromatic systems with good affinities. Given these similarities, **1** - **3** seemed ideal for investigating the effect of extended aromatic ancillary ligands on the DNA affinities and even binding mode of these hosts. Consequently, the water-soluble chloride salts of **2** and **3** were synthesized using reported methods and their interaction with duplex DNA in aqueous buffer solutions was compared to **1**.
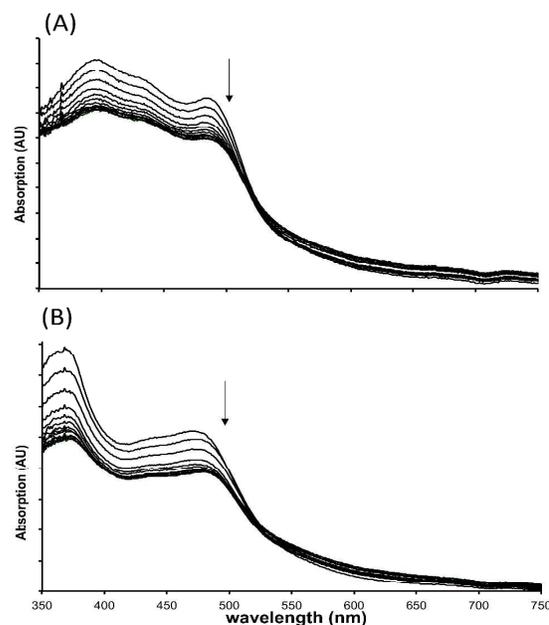

Fig. 2 Details of absorption spectra changes observed in aqueous solutions of macrocycle **2** (A) and **3** (B) on progressive addition of CT-DNA. Conditions: Conditions: [macrocycle] = 50 mM; buffer = 25 mM NaCl and 5 mM tris (pH 7.0) made with doubly distilled water (Millipore).

Initially, we investigated changes in absorption spectra induced by progressive addition of calf thymus DNA, CT-DNA –Fig. 2.

In both cases pronounced hypochromicity in bands above 300 nm were observed, however there are clear differences in the two hosts' optical responses. Changes observed in the MLCT bands of **2** are slightly lower than those reported for **1**: maxima at 395 nm and 485 nm show a reduction of 21% and 20.51% respectively (maximum change in **1** = 27%). In similar experiments with **3**, CT-DNA causes a comparable 23.85% hypochromic change in a band centred at 480 nm, although the band at 370 nm – which is associated with the dppz ligand – shows an appreciable larger reduction of 35.7%. This indicates that, for **3** at least, the ancillary ligands of the new hosts do interact directly with the DNA duplex. Given that the interaction of the Ru^{II}(dppz) moiety with DNA often

Fig. 3 Luminescence in aqueous solutions of macrocycle **2** (A) and **3** (B) before and after the addition of excess CT-DNA. Conditions: [macrocycle] = 30 mM; buffer = 25 mM NaCl and 5 mM tris (pH 7.0) made with doubly distilled water (Millipore). λ_{ex} = 440 nm

produces distinctive "light-switch" effects,[18,19] the luminescent responses of **2** and **3** to DNA were then investigated.

In contrast to our previous study, which revealed that the MLCT-centred luminescence of **1** decreases on progressive addition of CT-DNA (ie an "on-off" response), aqueous solutions of both **2** and **3** show DNA-induced luminescence *increases*. This is perhaps expected for **3** as it does contain the Ru^{II}(dppz) unit; however the increase in emission observed for **3** (162.6% at 649 nm) is actually lower than that for **2** (185.2% at 662 nm). In fact, DFT calculations on the **1 - 3** show that although the ancillary ligands do contribute to the MLCT excited states of the macrocycles, they are largely located on the qtpy bridging ligand, which explains why **3** does not show a full light-switch effect.[20]

However, the DNA-induced increase in the emission of **2** is more surprising, especially since it displays a *decrease* in emission when it binds bio-anions such as GTP and ATP. However, this response is consistent with our previous studies that have shown that emission changes caused by host-guest interactions are due to alterations in the torsion angles between aromatic rings in the bridging ligand. This structural change, caused by the accommodation of a guest within the macrocycle's binding pocket, can increase or decrease emission lifetimes (and hence intensity) by changing the rate of non-radiative decay processes.[21] It seems that, due to its increased rigidity compared to 2,2'-bpy, the addition of the phen ancillary ligand reduces flexibility of the binding pocket and changes how the macrocycle makes contact duplex DNA or, through
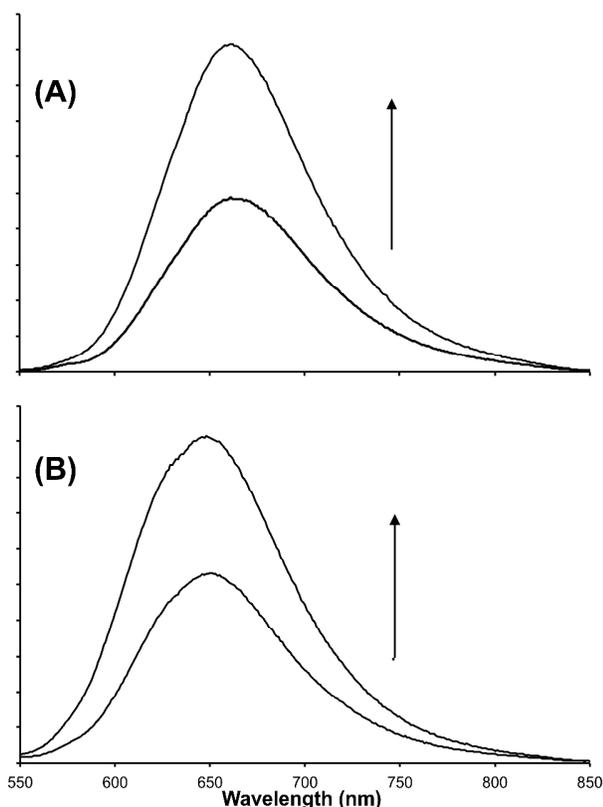
Table 1 CT-DNA binding parameters of macrocycles **1 – 3**[a]

Macrocycle	K_b/M^{-1}	n/bp
1[b]	3.3×10^6	1.1
2	8.8×10^5	4.7
3	4.4×10^5	1.4

[a]Estimated error ±10%. [b]Data from ref 16 included to aid comparisons.

The luminescent changes on addition of CT-DNA were used to construct saturation binding curves, which were then fitted to the commonly employed McGhee-von Hippel model for non-cooperative binding to an isotropic lattice.[22] This treatment produced the data summarised in Table 1. This data shows that although **3** contains extended ligands its binding affinity is almost an order of magnitude lower that **1**. More interestingly, while the affinity of **2** is slightly lower than **1**, its site size is appreciably
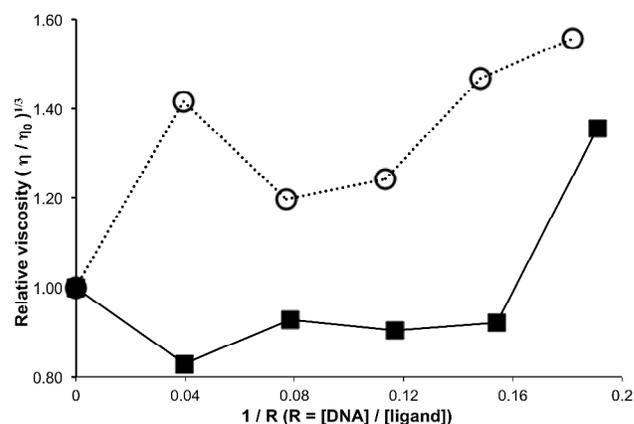

Fig. 4 Typical relative viscosity changes for CT-DNA on addition of **2** (■) or **3** (○). Conditions: 27 °C, 5 mM Tris buffer, 25 mM NaCl, pH 7.0.

larger, further illustrating how ancillary ligands can affect binding. Since the nature of the ancillary ligands clearly affects both optical output and DNA binding parameters of the macrocycles any possible change in binding mode were investigated through viscosity experiments.

Hydrodynamic-based method provides a direct and reliable assessment of reversible DNA modes. Intercalators increase the length and rigidity of a DNA helix and hence clearly produce an increase in the relative viscosity of DNA solutions, whereas classical groove binders induce no gross changes in DNA viscosity.[23] However, substrates like **1** that kink or bend DNA produce viscosity decreases at low mixing ratios followed by

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increases at higher ratios; this is thought to occur because increasing unphased kinks introduce a rigid superhelical structure.[24] Typical data for such experiments on **2** and **3** are shown Fig. 4. As can be seen, **2** induces viscosity changes that are indicative of large DNA scale bending and are similar to those previously reported for **1**. Given the large negative changes in viscosity at low macrocycle loading ratios it is possible that both **1** and **2** can condense DNA, suggesting that they could be of potential interest as vectors for gene delivery.[25] This possibility is currently under investigation.

In contrast, macrocycle **3** solely induces increases in viscosity. These data provide direct evidence for an intercalative DNA binding mode for macrocycle **3**. As far as we are aware, this is the first example of DNA intercalation by a self-assembled macrocycle and demonstrates that dppz ancillary ligands have a profound effect on the binding properties of the central macrocyclic platform.

In conclusion, this study illustrates how the photophysical and biophysical properties of a self-assembled macrocyclic core that targets biomolecular substrates can be modulated through judicious selection of ancillary ligands. In the future, this concept will be used to further modulate the behaviour of this system so that specific bio-targets and functions can be selected, these studies will provide the basis of future reports.

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