Supporting Information for:

Unexpected DNA binding properties with correlated downstream biological applications in mono vs. bis-1,8-naphthalimide Ru(II)-polypyridyl conjugates

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Figure S1 A) $^1$H NMR (400 MHz, CD$_3$CN) of Ru.Nap and B) $^1$H NMR (400 MHz, CD$_3$OD) of Ru.2Nap showing the aromatic regions.
Figure S2 $^{13}$C NMR (150 MHz, CD$_3$CN) of Ru.Nap.

Figure S3 DEPT 135 NMR (150 MHz, CD$_3$CN) of Ru.Nap.
Figure S4 $^{13}$C NMR (100 MHz, CD$_3$OD) of Ru.2Nap.
Figure S5 HH COSY spectrum (600 MHz, CD$_2$OD) of the aromatic region of Ru$_2$Nap.
Figure S6 CH COSY spectrum (600 MHz, CD₃OD) of Ru₂Nap.
Figure S7 Long range CH COSY spectrum (600 MHz, CD$_3$OD) of Ru.2Nap.
Figure S8 Excitation spectra (\(\lambda_{em} 670\) nm) of Ru (——) and Ru.2Nap (——), both at 6.5 \(\mu\)M in 10 mM phosphate buffer, at pH 7.

Figure S9 Representation of the antenna effect in which the excited state energy of the 1,8-naphthalimides is transferred to the Ru(II) MLCT excited state.
ΔΓ = - [(E_{1/2} Naph/Naph^{*} - E_{1/2} Ru^{3+/2+})] – E^*

**Equation S1** Simplified Rehm-Weller equation, from which a driving force for electron transfer for Ru.Nap and Ru.2Nap was determined. Where E^* is the excitation energy of the vibrationally relaxed MLCT excited state, estimated from the luminescence maximum.

**Figure S10** (a) Changes in the MLCT emission spectrum of Ru.Nap (6.5 μM) (λ_{ex} 450 nm) upon addition of st-DNA (0 – 130 μM base pairs) in 10 mM phosphate buffer, at pH 7. (b) The change in integrated MLCT emission intensity as a function of Bp/D.

**Figure S11** (a) Changes in the MLCT emission spectrum of Ru.Nap (6.5 μM) (λ_{ex} 338 nm) upon addition of st-DNA (0 – 130 μM base pairs) in 10 mM phosphate buffer, at pH 7. (b) The change in integrated MLCT emission intensity as a function of Bp/D.
Figure S12 Changes in the MLCT emission spectrum of Ru.2Nap (6.5 μM) (λ_{ex} 450 nm) upon addition of st-DNA (0 – 29.25 μM base pairs) in 10 mM phosphate buffer, at pH 7. Inset: The change in integrated MLCT emission intensity as a function of Bp/D.

Figure S13 Excitation spectrum of Ru.2Nap (6.5 μM) (λ_{em} 670 nm) in 10 mM phosphate buffer, at pH 7 in the absence (---) and presence of st-DNA at a Bp/D ratio of 0.6 (----) and 4.5 (----).
**Figure S14** Circular dichroism curves of (a) st-DNA (150 μM) in 10 mM phosphate buffer, at pH 7 in the absence and presence of Ru.2Nap at varying ratios, and (b) the difference spectra obtained.

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<thead>
<tr>
<th>Lane</th>
<th>% Form I</th>
<th>% Form II</th>
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**Table S1** Percentage of Form I vs II pBR322 plasmid DNA from the cleavage study in Fig. 11. Lane 1: Plasmid DNA control; Lane 2: Ru(bpy)$_2^{2+}$ (Bp/D 5) 5 min irradiation; Lanes 3-5: Ru.Nap (Bp/D 5) 1, 3, 5 min respectively; Lanes 6-8: Ru.2Nap (Bp/D 5) 1, 3, 5 min respectively. Determination of the relative intensity of the bands showed that DNA was somewhat damaged to begin with, being comprised of 73% Form I and 27% Form II.
Figure S15 Confocal Laser Scanning Microscopy live cell images of Ru.2Nap (30 µM) with HeLa cells. Shown are the images obtained with (A) the bright field view of treated cells after 4 hrs incubation, stained with DAPI (blue) and Ru.2Nap (red), (B) overlay of Ru.2Nap (red) and nuclear co-stain DAPI (blue), (C) Ru.2Nap emission alone (red), (D) the bright field view of treated cells after 24 hrs incubation, stained with DAPI (blue) and Ru.2Nap (red), (E) overlay of Ru.2Nap (red) and nuclear co-stain DAPI (blue), (F) Ru.2Nap emission alone (red).