Electronic Supplementary Material (ESI) for Dalton Transactions.
This journal is © The Royal Society of Chemistry 2017

Evidence for interstrand DNA covalent binding of two dinuclear Ru(II) complexes. Influence of the extra ring of the bridging ligand on the DNA interaction and cytotoxic activity

Héctor J. Lozano, Natalia Busto, Gustavo Espino, Arancha Carbayo, José M. Leal, James A. Platts, and Begoña García,

Supporting Information.

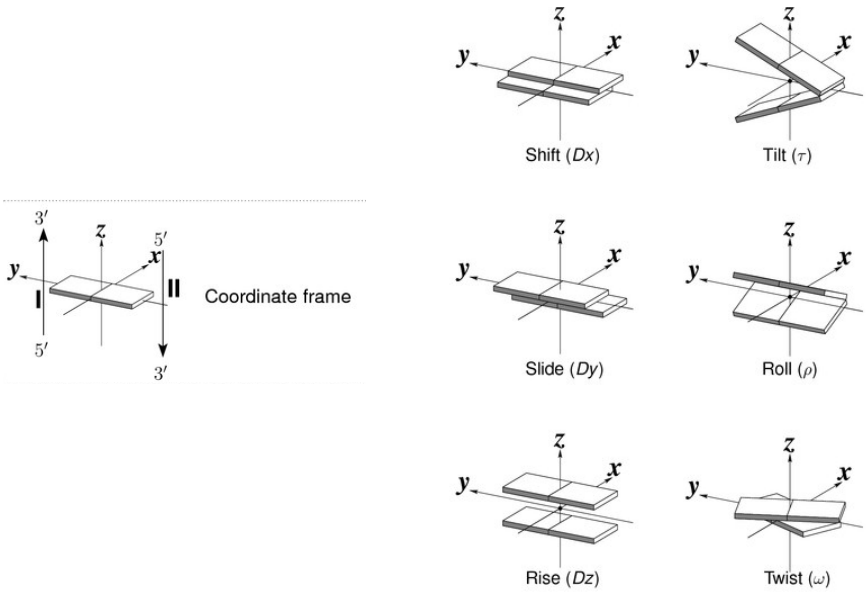


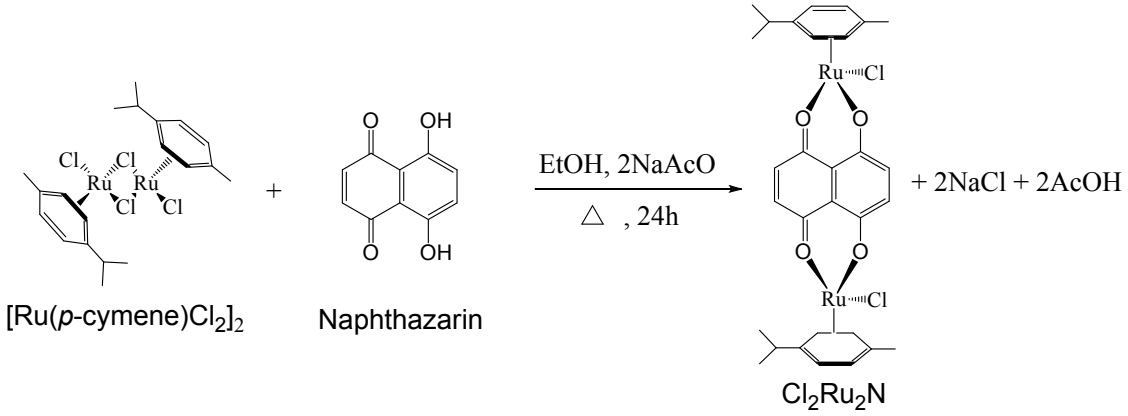
Fig. S1. DNA local base-pair step parameters representation. Shadowed sides represent view from minor groove. Image taken from X3DNA publication.[44]

Synthesis of $[(\eta^6\text{-}p\text{-cymene})_2Ru_2(OO\cap OO)(Cl)_2]$ complexes.

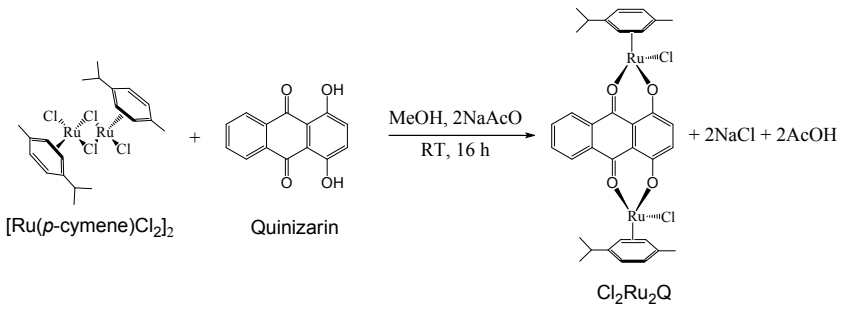
$[\eta^6$- (*p*-cymene)$_2$Ru$_2$(5,8-dihydroxy-1,4-naphthoquinonato)(Cl)$_2$], **Cl_2Ru_2N:** In a Schlenk flask $[Ru(p\text{-cym})Cl_2]_2$ (0.236 mmol) and Naphthazarin (0.236 mmol) were added to 10 mL of deoxygenated ethanol under N_2 athmosphere, giving a red-coloured solution. Then, Sodium Acetate (AcONa, 0,472 mmol) was added to the previous solution. The mixture was stirred and heated under reflux overnight, giving a brown suspension which was filtered, obtaining a brown solid. Then, the solid was washed with ether (3×10 mL) and hexane (3×10 mL) and dried under vacuum to yield the final product. The reaction is outlined in Figure SI 2A. **Characterization**: ^{1}H NMR (400 MHz, $CDCl_3$, 25°C): δ 8.35 (dd, *J* = 6.0, 3.3 Hz, 2H), 7.62 (dd, *J* = 6.0, 3.4 Hz, 2H), 7.02 (d, *J* = 0.8 Hz, 2H), 5.55 (dd, *J* = 17.6, 5.7 Hz, 4H), 5.32 – 5.23 (m, 4H), 2.95 (dt, *J* = 13.9,

6.9 Hz, 2H), 2.26 (s, 6H), 1.59 (s, 3H), 1.42 – 1.32 (m, 13H). Elemental analysis calculated for $C_{30}H_{32}O_4Cl_2Ru_2$: C, 49.39; H 4.42; N, O,and S, 0%.

[(η^6-*p*-cymene)$_2$Ru$_2$(1,4-dihydroxyanthraquinonato)(Cl)$_2$], **Cl$_2$Ru$_2$Q:** in a Schlenk flask [Ru(*p*-cym)Cl$_2$]$_2$ (0.326 mmol) was added to 30mL methanol. Then, Quinizarin (0.326 mmol) and AcONa (0.652 mmol) were added under N_2 atmosphere to the previous solution. The reaction was stirred overnight at room temperature, giving a green suspension which was filtered, giving a green solid which was washed with CH_2Cl_2, hexane and dried under vacuum, yielding the final product, Figure SI 2B. **Characterization:** ^{1}H NMR (400 MHz, $CDCl_3$, 25 ºC): δ 6.95 (s, 4H), 5.49 (d, *J* = 6.1 Hz, 4H), 5.23 (d, *J* = 6.0 Hz, 4H), 2.92 – 2.80 (m, 2H), 2.22 (s, 6H), 1.31 (d, *J* = 6.9 Hz, 12H) ppm.). Elemental analysis calculated for $C_{34}H_{34}O_4Cl_2Ru_2$: C, 52. 38; H,4.4; N, O and S,0%.



A



B

Fig. S2. Synthesis of (A) Cl_2Ru_2N and (B) Cl_2Ru_2Q from [Ru(p-cym)Cl_2]$_2$ and Naphthazarin and Quinazarin, respectively.

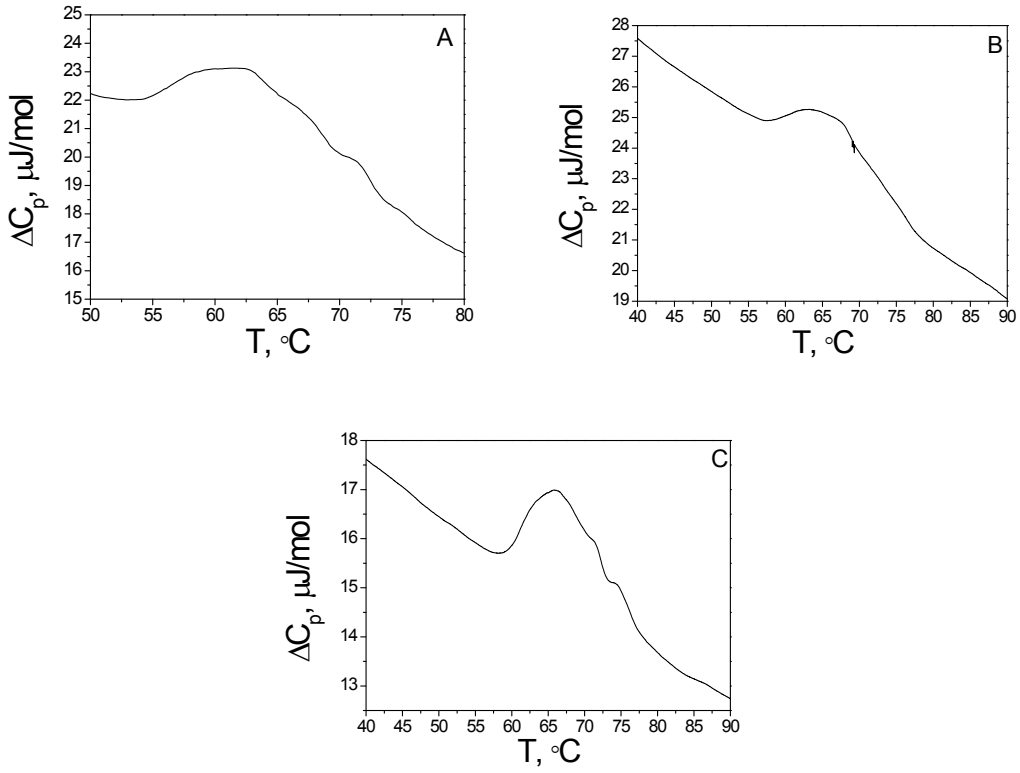


Fig. S3. DSC curves for (A) free ctDNA, (B) Naphthazarin/ctDNA system at $C_D/C_P = 0.2$ and (C) Quinizarin/ctDNA system at $C_D/C_P = 0.2$. $C_P = 4.04 \times 10^{-4}$ M, scan rate:1 °C/min I = 6.5 mM ($NaClO_4$) and pH = 7.

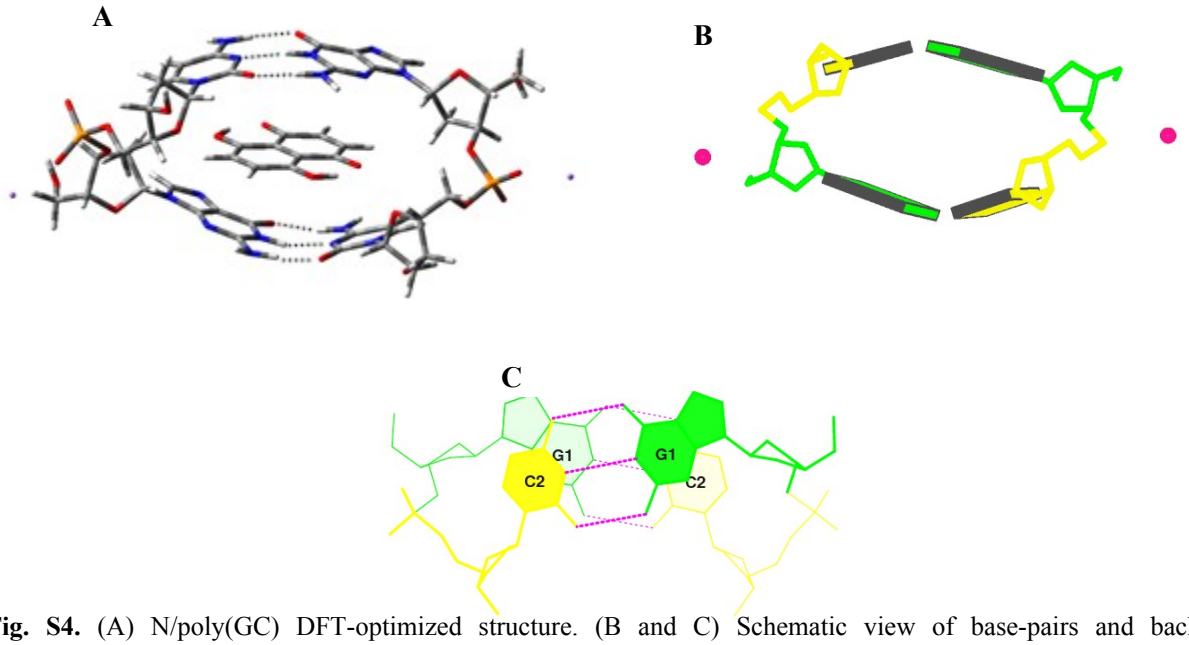


Fig. S4. (A) N/poly(GC) DFT-optimized structure. (B and C) Schematic view of base-pairs and backbone conformation from minor groove view and top view respectively. Green and yellow stand for Guanine and Cytosine molecules, respectively.

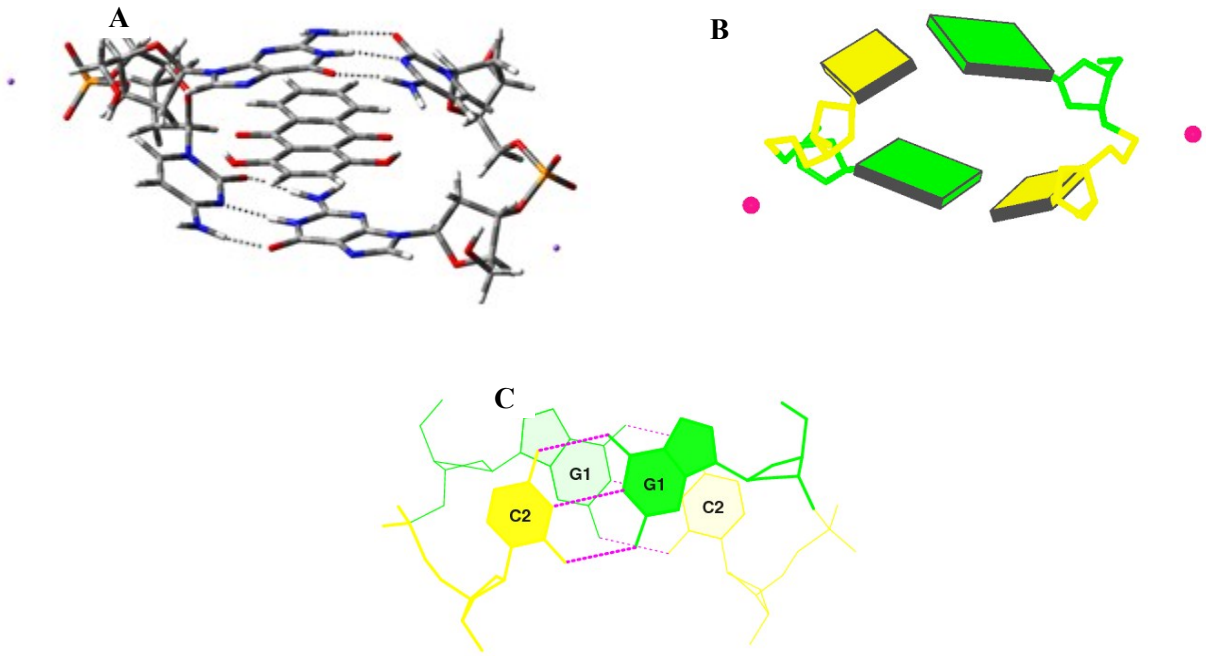


Fig. S5. (A)Q/poly(GC) DFT-optimized structure. (B and C) Schematic view of base-pairs and backbone conformation from minor groove view and top view respectively. Green and yellow stand for Guanine and Cytosine molecules, respectively.

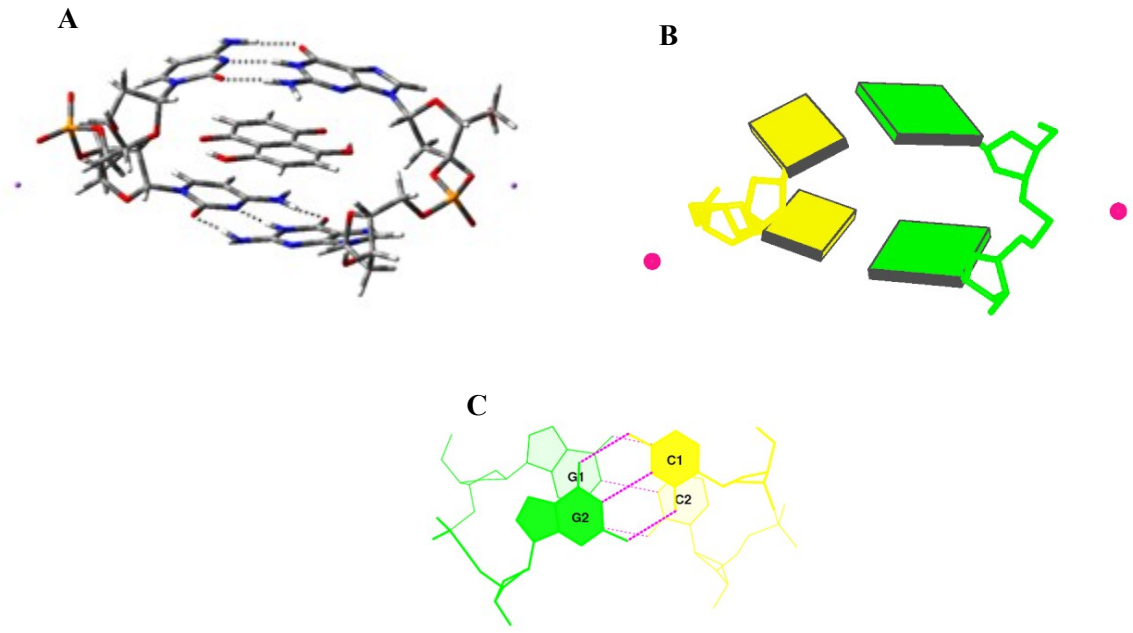


Fig. S6. (A)N/poly(G)·poly(C) DFT-optimized structure. (B and C) Schematic view of base-pairs and backbone conformation from minor groove view and top view, respectively. Green and yellow stand for Guanine and Cytosine molecules, respectively.

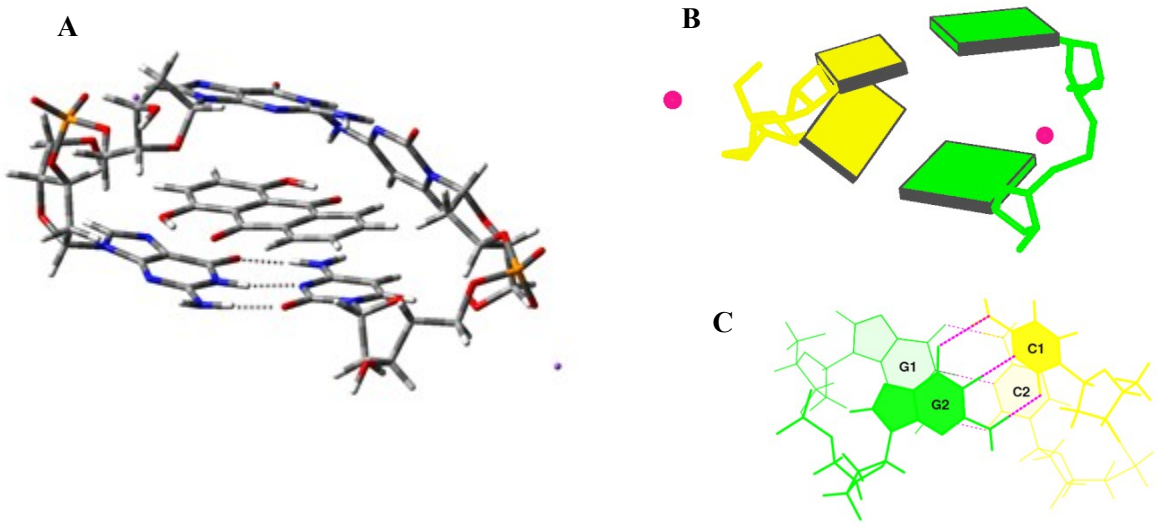


Fig. S7. (A)Q/poly(G)·poly(C) DFT-optimized structure. (B and C) Schematic view of base-pairs and backbone conformation from minor groove view and top view respectively. Green and yellow stand for Guanine and Cytosine molecules, respectively.

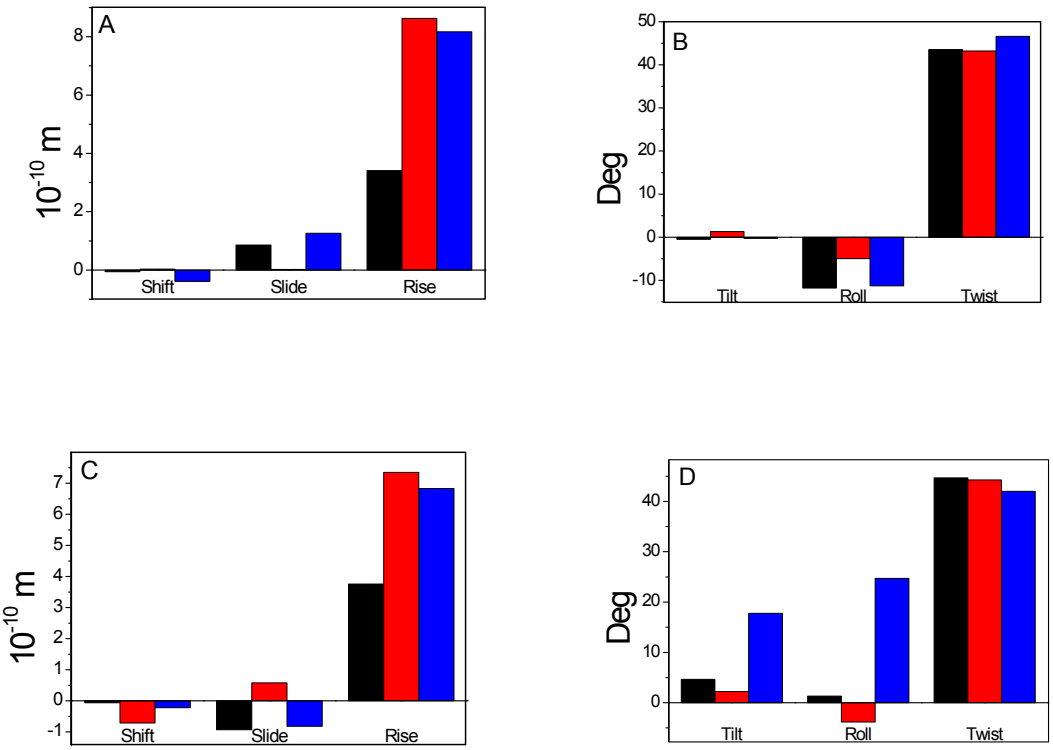


Fig. S8. DNA parameter values obtained for (A, B): free poly(GC) (—), N/poly(GC) (—) and Q/poly(GC) (—) and for (C, D) free poly(G)·poly(C) (—), N/ poly(G)·poly(C) (—) and Q/ poly(G)·poly(C) (—). Roll parameter for Q in Q/poly(G)·poly(C) system is significantly higher than that for free poly(G)·poly(C) and significantly differs from the negative value of N/poly(G)·poly(C) system. Similar behaviour is obtained for slide parameter. Furthermore, for poly(GC) systems Roll parameter diminishes in the presence of N but it remains the same in the presence of Q. By contrast, Slide parameter is negligible in presence of N, meaning that there is no displacement of base-pairs along the y axis, whereas in the presence of Q Slide parameter is higher than that obtained for free poly(GC).

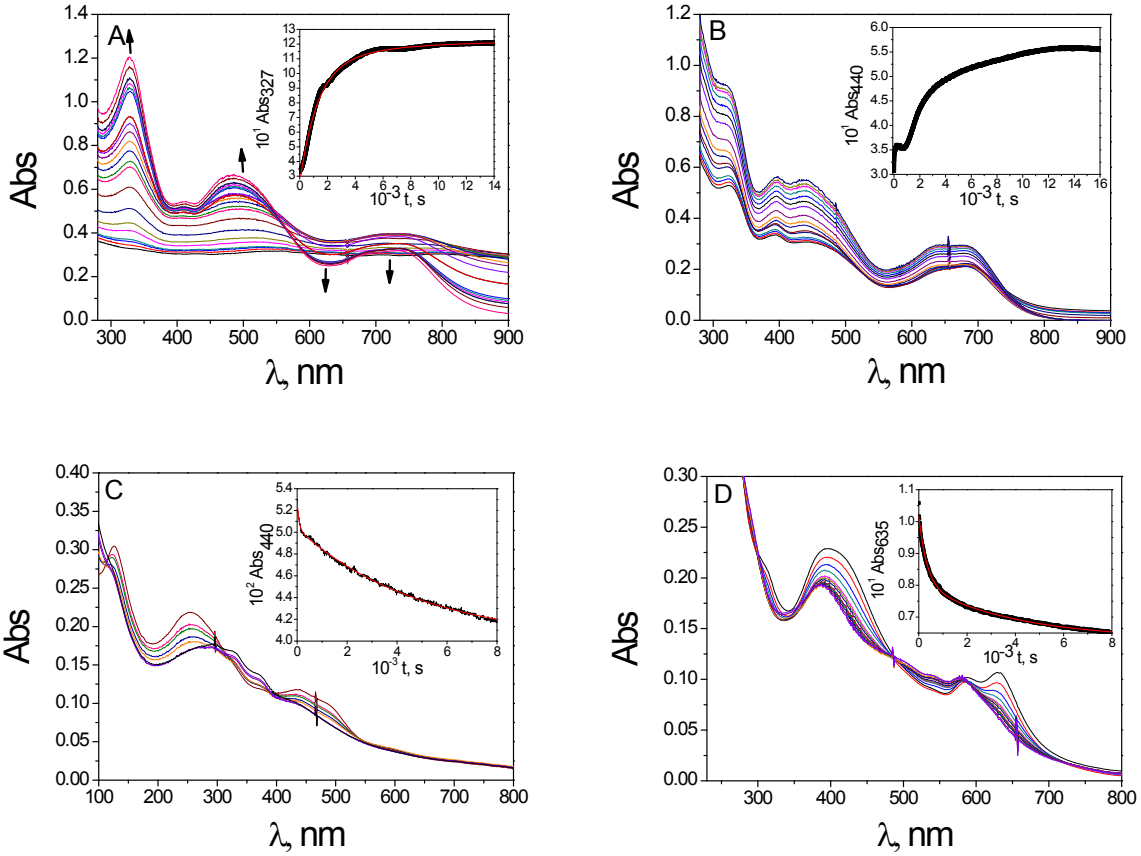


Fig. S9. **[**$(DMSO)_2Ru_2X]^{2+}$ formation from Cl_2Ru_2X. (A) X = N and (B) X = Q: Absorbance spectra as function of time. Insets: Absorbance-time plot. $C_D = 1.5 \times 10^{-5}$ M, 100% DMSO and T = 25°C. $[(H_2O)_2Ru_2X]^{2+}$ formation from $[(DMSO)_2Ru_2X]^{2+}$. (C) X = N and (D) X = Q: Absorbance spectra as function of time. Insets: Absorbance-time plot. Continuous red lines represent the fitting to biexponential functions of the data pairs. $C_D = 2.5 \times 10^{-5}$ M, I = 6.5 mM ($NaClO_4$). pH = 7 and T = 25 °C.

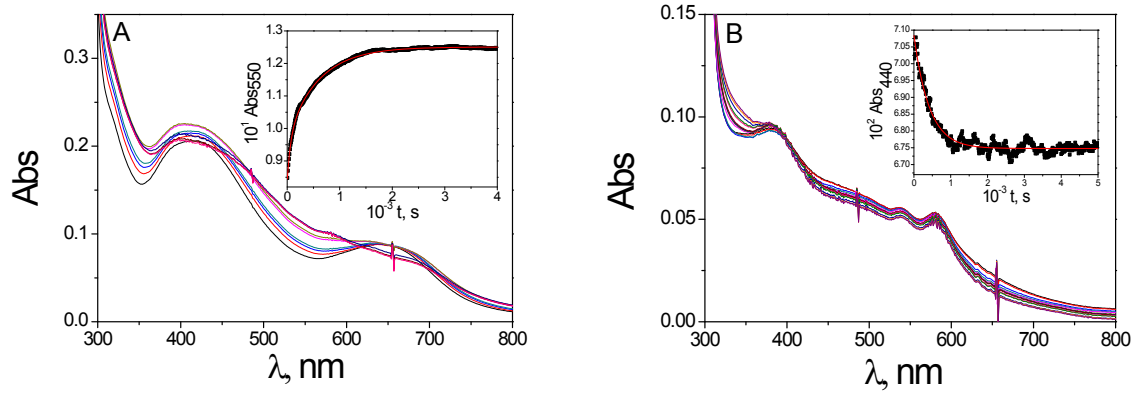


Fig. S10. Absorbance spectra as a function of time for (A) $[(H_2O)_2Ru_2N]^{2+}$/*d*GMP system, $C_D = 5.0 \times 10^{-5}$M and (B) $[(H_2O)_2Ru_2Q]^{2+}$/*d*GMP system, $C_D = 2.5 \times 10^{-5}$M. Insets: Absorbance-time plots. Continuous red lines represent the fitting to biexponential funtions of the data pairs. $C_P/C_D = 10$, I = 6.5 mM ($NaClO_4$), pH = 7 and T = 25°C.

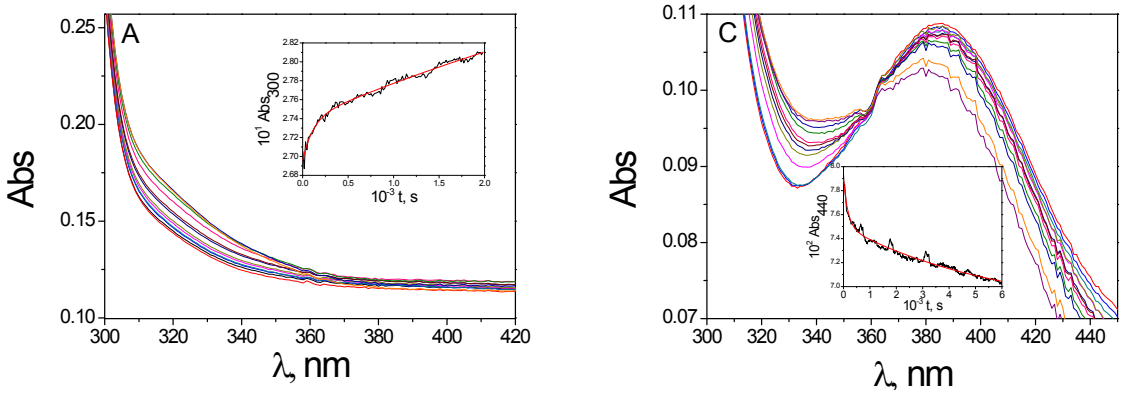


Fig. S11. Absorbance spectra as a function of time for: (A) $[(H_2O)_2Ru_2N]^{2+}$/ctDNA system, $C_D = 5.0 \times 10^{-5}$ M and (B) $[(H_2O)_2Ru_2Q]^{2+}$/ctDNA system $C_D = 2.5 \times 10^{-5}$ M. Insets: absorbance *versus* time plot. Continuous red lines represent the fitting to biexponential funtions of the data pairs. $C_P/C_D = 10$, I = 6.5 mM ($NaClO_4$), pH = 7 and T = 25 °C.

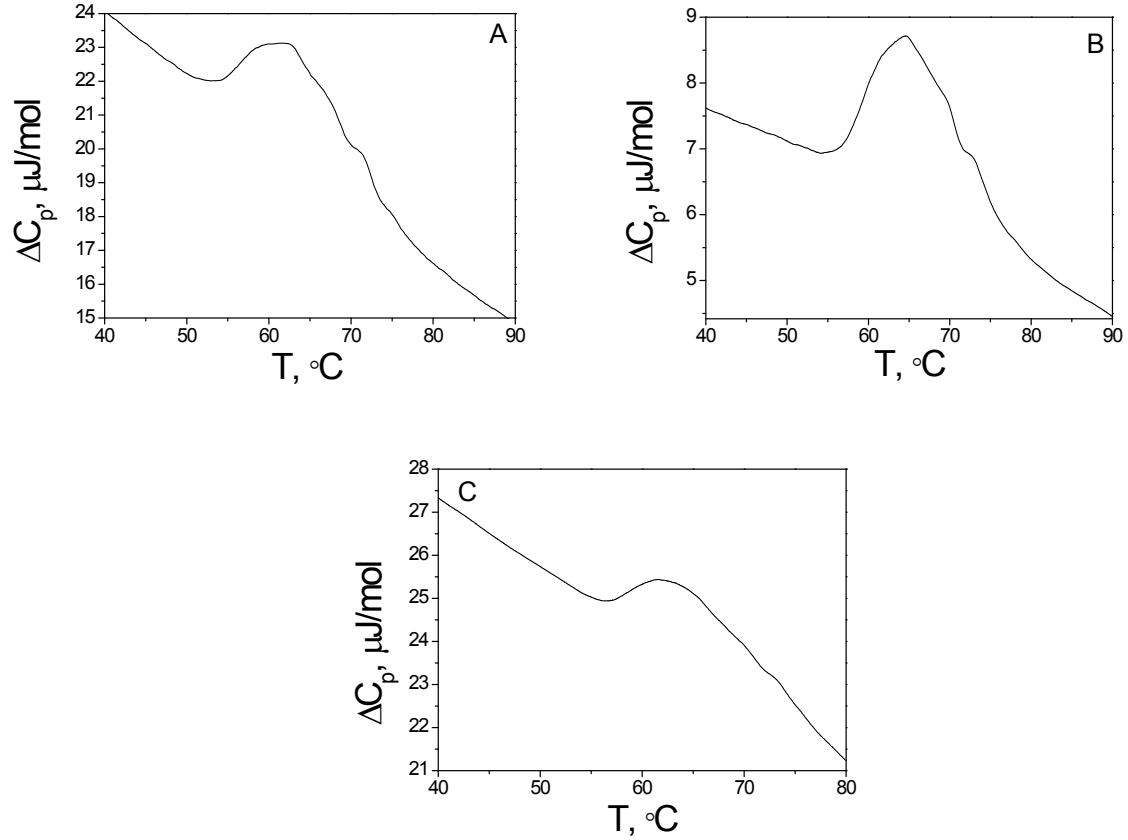


Fig. S12. DSC curves for $[(H_2O)_2Ru_2X]^{2+}$/ctDNA systems. (A) Free ctDNA, (B) $[(H_2O)_2Ru_2N]^{2+}$/ctDNA, $C_D/C_P = 0.1$, (C) $[(H_2O)_2Ru_2Q]^{2+}$/ctDNA. $C_D/C_P = 0.1$. $C_P = 4.04 \times 10^{-4}$ M, scan rate:1°C/min, I = 6.5 mM ($NaClO_4$) and pH = 7.

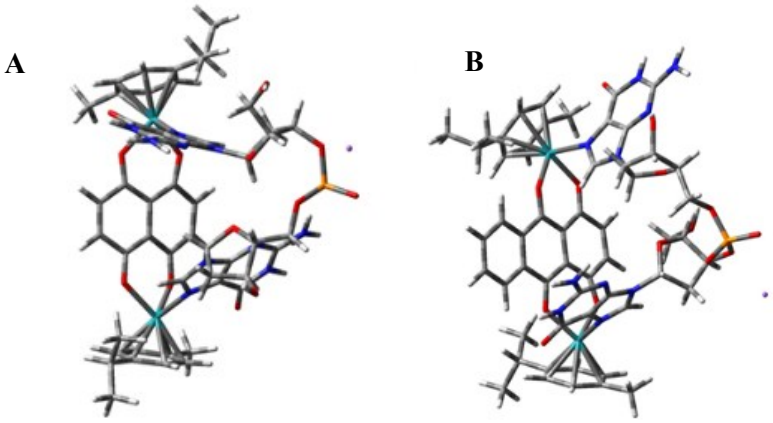


Fig. S13. (A) $[(H_2O)_2Ru_2N]^{2+}$/poly(G) and (B) $[(H_2O)_2Ru_2Q]^{2+}$/poly(G) DFT-optimized structures.

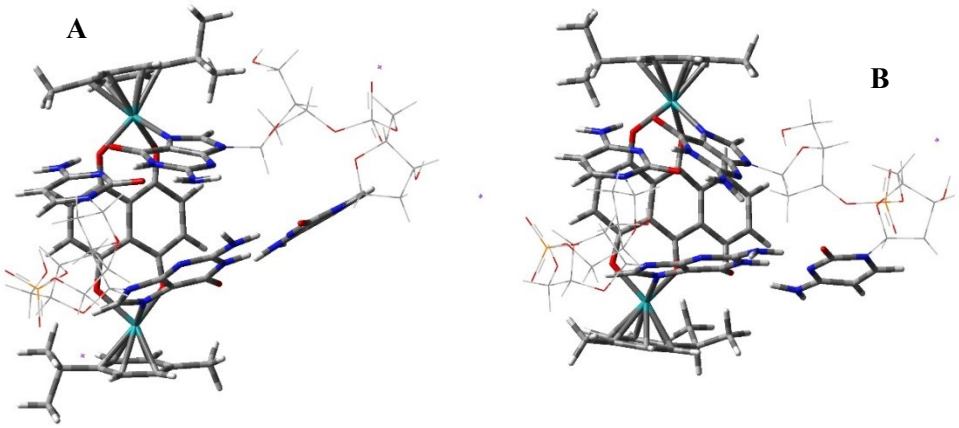


Fig. S14. (A) $[(H_2O)_2Ru_2N]^{2+}$/poly(GC) and (B) $[(H_2O)_2Ru_2Q]^{2+}$/poly(GC) DFT-optimized structures. Backbone is represented as wireframe for a better visualization.

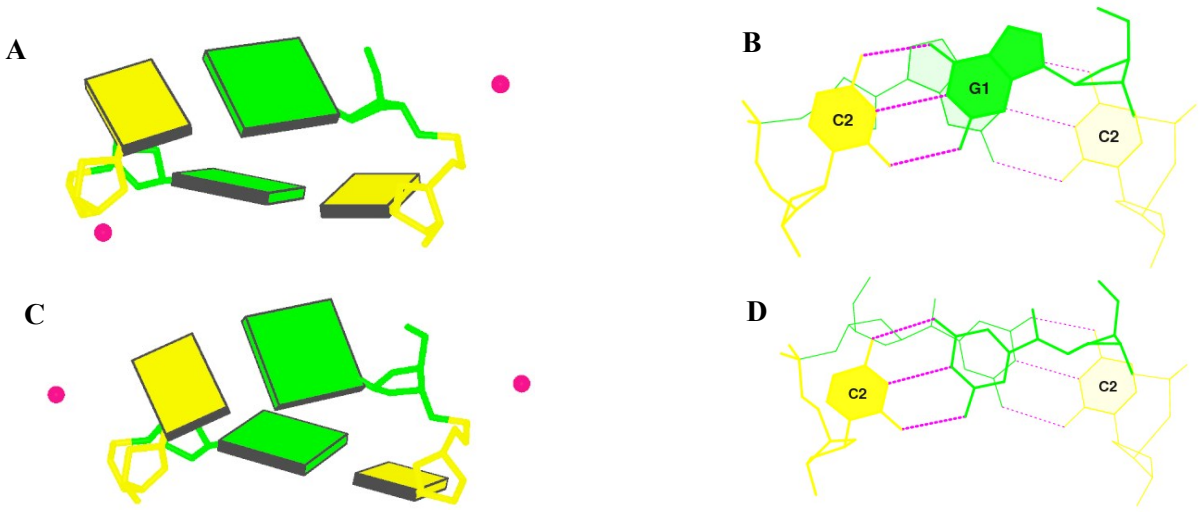


Fig. S15. (A) and (B) Schematic view of base-pairs and backbone conformation from minor groove view and top view respectively for $[(H_2O)_2Ru_2N]^{2+}$/poly(GC). (C) and (D) Schematic view of base-pairs and backbone conformation from minor groove view and top view respectively for $[(H_2O)_2Ru_2Q]^{2+}$/poly(GC). Green and yellow squares stand for Guanine and Cytosine molecules respectively.

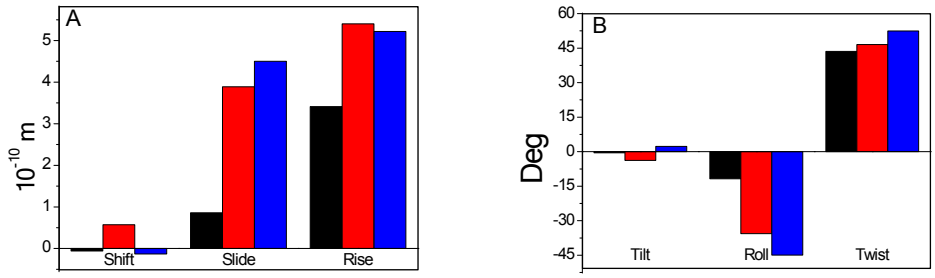


Fig. S16. DNA parameter values obtained for (—) free poly(GC), (—) $[(H_2O)_2Ru_2N]^{2+}$/poly(GC) and (—) $[(H_2O)_2Ru_2Q]^{2+}$/poly(GC). (A) Shift, Slide and Rise and (B) Tilt, Roll and Twist.

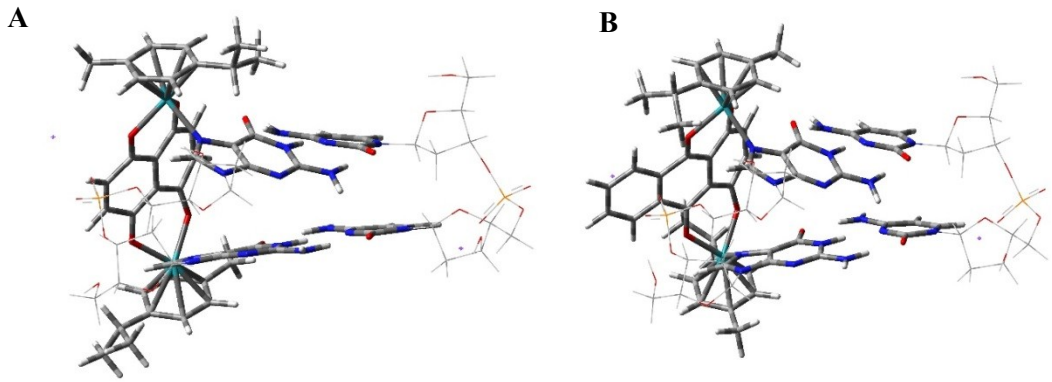


Fig. S17. (A) $[(H_2O)_2Ru_2N]^{2+}$/poly(G)·poly(C) and (B) $[(H_2O)_2Ru_2Q]^{2+}$/poly(G)·poly(C) DFT-optimized structures. Backbone is represented as wireframe for a better visualization.

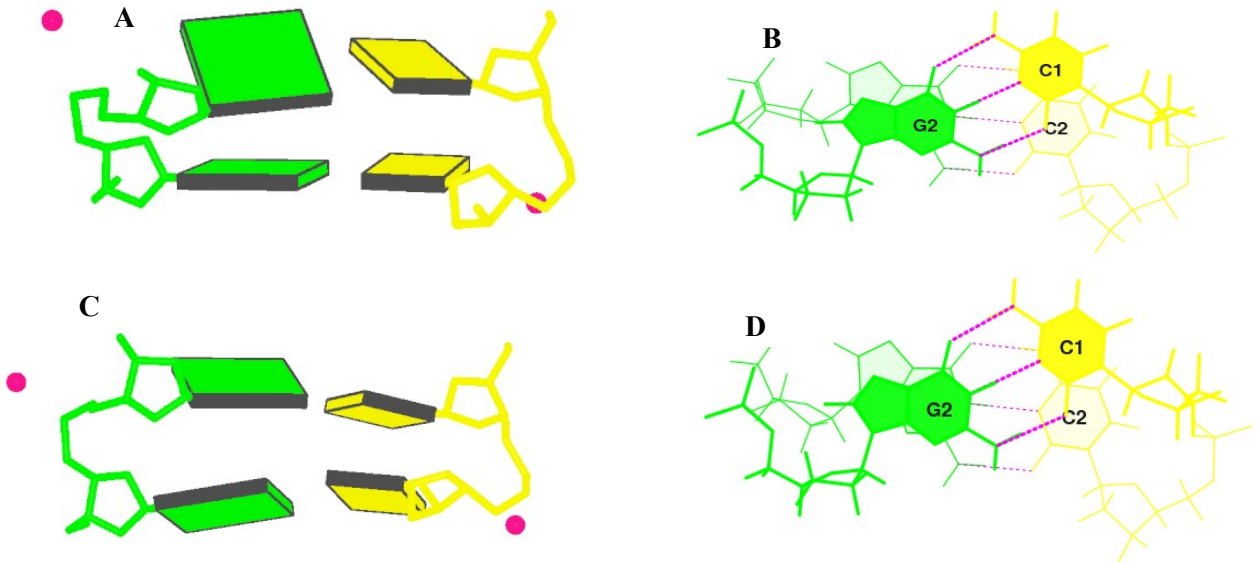


Fig. S18. (A)and (B)Schematic view of base-pairs and backbone conformation from minor groove view and top view respectively for $[(H_2O)_2Ru_2N]^{2+}$/poly(G)·poly(C). (C)and (D): Schematic view of base-pairs and backbone conformation from minor groove view and top view respectively for $[(H_2O)_2Ru_2Q]^{2+}$/poly(G)·poly(C).Green and yellow stand for Guanine and Cytosine molecules respectively.

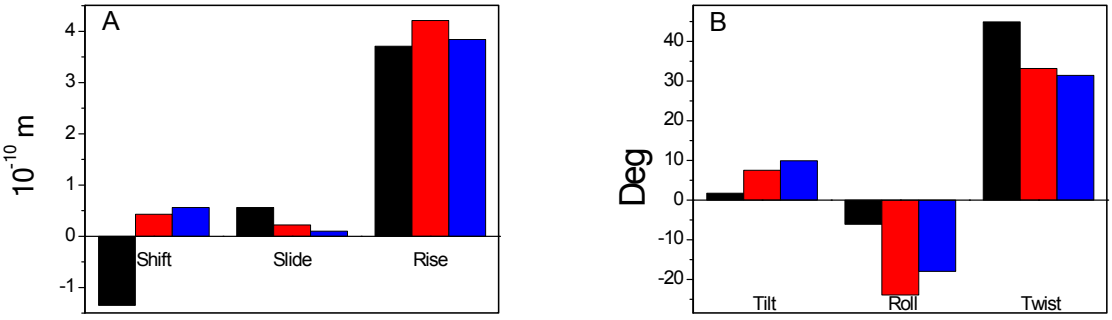


Fig. S19. DNA parameter values obtained for free (—) poly(G)·poly(C), (—) $[(H_2O)_2Ru_2N]^{2+}$/poly(G)·poly(C) and (—) $[(H_2O)_2Ru_2Q]^{2+}$/poly(G)·poly(C). (A) Shift, Slide and Rise and (B) Tilt, Roll and Twist.