# Synthetic and structural studies of ruthenium complexes as DNA major groove binders

**Tayler D. Prieto Otoya[1], Kane McQuaid[1], Neil G. Paterson[2], David J. Cardin[1], Georgia Menounou[3], Andrew Kellett[3], Christine J. Cardin[1].**

[1]Department of Chemistry, University of Reading, Whiteknights, RG6 6AD, UK; [2]Diamond Light Source, Didcot, Oxfordshire, OX11 0DE, UK; [3]School of Chemical Sciences, DCU, Ireland.

## Background

- Ruthenium polypyridyl complexes are of interest for their application as cellular probes, and as anticancer therapeutic agents.
- Understanding the structural factors that determine the affinity and selectivity in binding small molecules, in particular ruthenium complexes, towards DNA, would be valuable in the rational design of molecules that target specific sequences in the DNA.
- All previous crystallographic work had shown intercalative binding from the DNA minor groove[1].
- One rationale for establishing that it is possible to bind a ruthenium polypyridyl complex in the DNA major groove is to enable structure-based design of a chemical nuclease (modified-TFOs for instance).

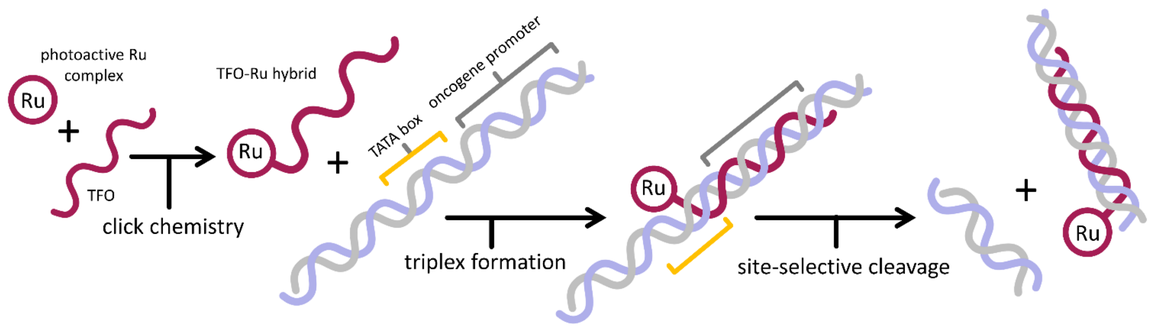


**Fig. 1** Schematic showing assembling of a major groove intercalating Ru complex, linked by "click-chemistry" to a triplex forming oligonucleotide.

## Results

- Our crystal structure provides the first crystallographic evidence that a ruthenium polypyridyl complex can bind in the DNA major groove.

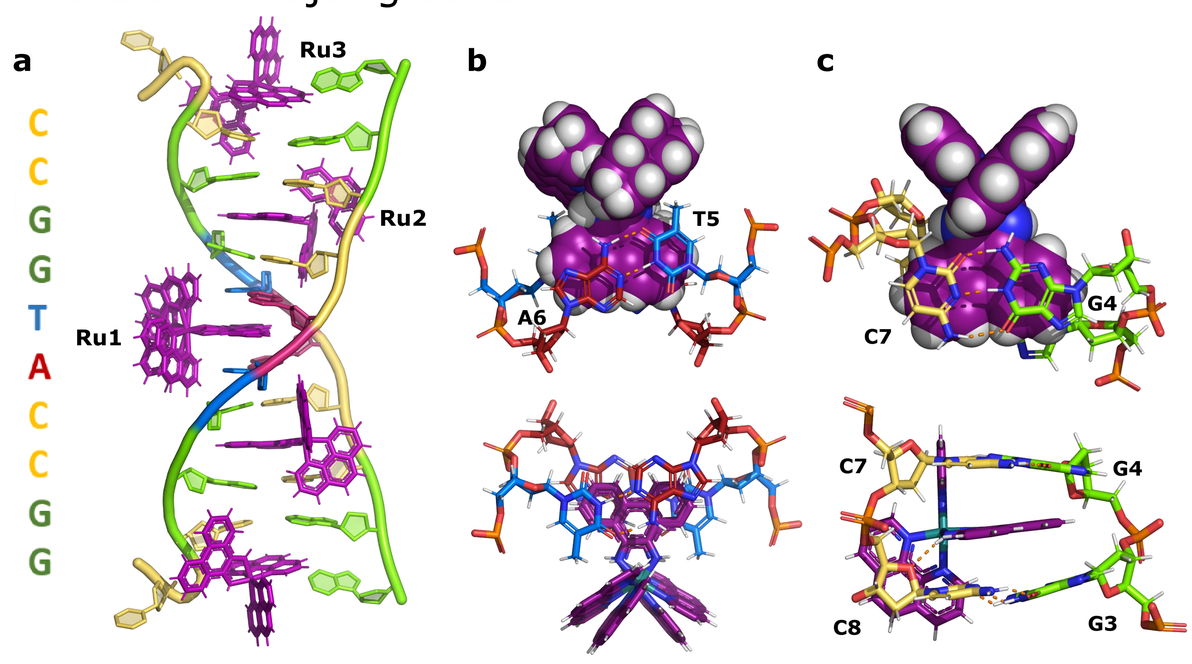


**Fig. 4** a) DNA sequence used in the X-ray work with the completed structure assembly; b) projection of the central TA/TA step showing symmetrical intercalation from the major groove; c) projection of one flanking GG/CC step showing angle (canted) intercalation from the minor groove.

- Different interactions are observed in the minor groove, where a hydrogen-bonding between the C8-sugar and a hydrogen of the Ru(II) diimine complex is formed.

## Crystallisation

- Crystals of $[Ru(phen)_2phi]^{2+}$, a major groove binder[2], were obtained by vapour diffusion of diethyl ether into a saturated solution of the complex in acetonitrile. Dark red needles were obtained.

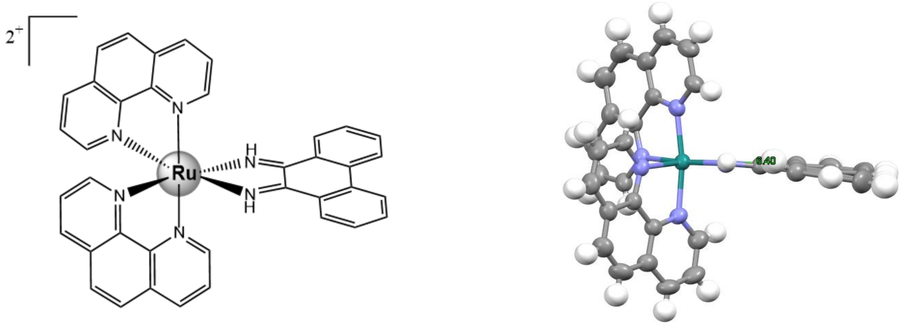


**Fig. 2** Crystal structure; a) line drawing of Λ-$[Ru(phen)_2phi]^{2+}$; b) Mercury plot of Λ-$[Ru(phen)_2phi]^{2+}$where bending of 6.4° of the 9,10-phenanthrenequinonediamine (phi) ligand is observed.

- The successful combination in the crystallisation experiment was that of the DNA decamer sequence $d(CCGGTACCGG)_2$, with the lambda enantiomer: Λ-$[Ru(phen)_2phi]^{2+}$.

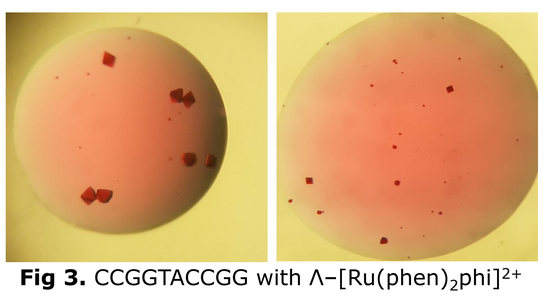

**Fig 3.** CCGGTACCGG with Λ–$[Ru(phen)_2phi]^{2+}$

- Data collection was obtained at Diamond Light Source (DLS), in collaboration with Dr. Neil G. Paterson.

## Thermal analysis

- Melting temperature experiments were used to identify specific Ru(II) complexes that selectively recognised TATA rich sequences found in the promoter regions of cancer-causing genes.

| Sequence (12mer) | $T_M$ (℃) | $T_M$ (℃) Δ-$[Ru(phen)_2phi]^{2+}$ | Δ-$T_M$ | $T_M$ (℃) Λ-$[Ru(phen)_2phi]^{2+}$ | Λ-$T_M$ |
|---|---|---|---|---|---|
| (1) $d(ATCGGCGCCGAT)_2$ | 67.76 | 71.78 | +4.01 | 74.71 | +6.95 |
| (2) $d(ATMGGMGMMGAT)_2$ | 72.47 | 78.56 | +6.09 | 82.01 | 9.54 |
| (3) $d(GCCGGTACCGGC)_2$ | 69.52 | 73.32 | +3.80 | 77.54 | +8.02 |
| (4) $d(GCCGGATCCGGC)_2$ | 66.27 | 70.33 | +4.06 | 73.64 | +7.38 |
| (5) $d(GCTTTATAAAGC)_2$ | 41.68 | 49.58 | +7.90 | 66.79 | +25.11 |
| (6) $d(GCUUUAUAAAGC)_2$ | 37.67 | 42.90 | +5.23 | 63.27 | +25.60 |

**Table 1.** Thermal melting ($T_M$) analysis of resolved $[Ru(phen)_2phi]^{2+}$ with specific oligomers (experiments conducted at pH 7.4 at *r* loading of 0.5).M=Methyl, dC; U=Uracil.

- Λ-$[Ru(phen)_2phi]^{2+}$ exhibits high thermal stabilisation (+25 °C) of the TATA palindrome (sequence 5), over 4 times that obtained for the Δ enantiomer.

## Conclusion

- To our knowledge this is the first example of a metal complex in which the preferred groove for binding is sequence dependent. This knowledge can now be exploited in the design of sequence specific binding assemblies such as those containing TFOs.

This project (NATURE-ETN) has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 861381.

**References**

1. Cardin, C. J., Kelly, J. M. & Quinn, S. J. Photochemically active DNA-intercalating ruthenium and related complexes-insights by combining crystallography and transient spectroscopy. *Chem. Sci.* 8, 4705–4723 (2017).
2. Pyle, A. M. *et al.* Mixed-Ligand complexes of ruthenium(II): Factors governing binding to DNA. *J. Am. Chem. Soc.* **111**, 3051–3058 (1989).