A Crystallographic Study of Ruthenium Complexes Bound to DNA Reveals Sequence Specific Binding with a Directional Preference

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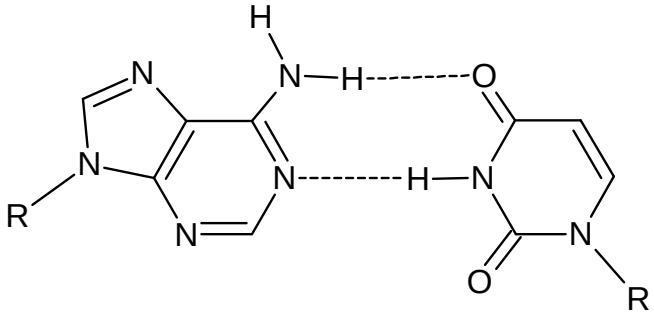
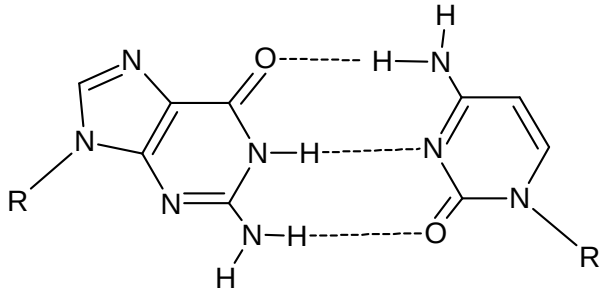
Diamond Light Source

DNA

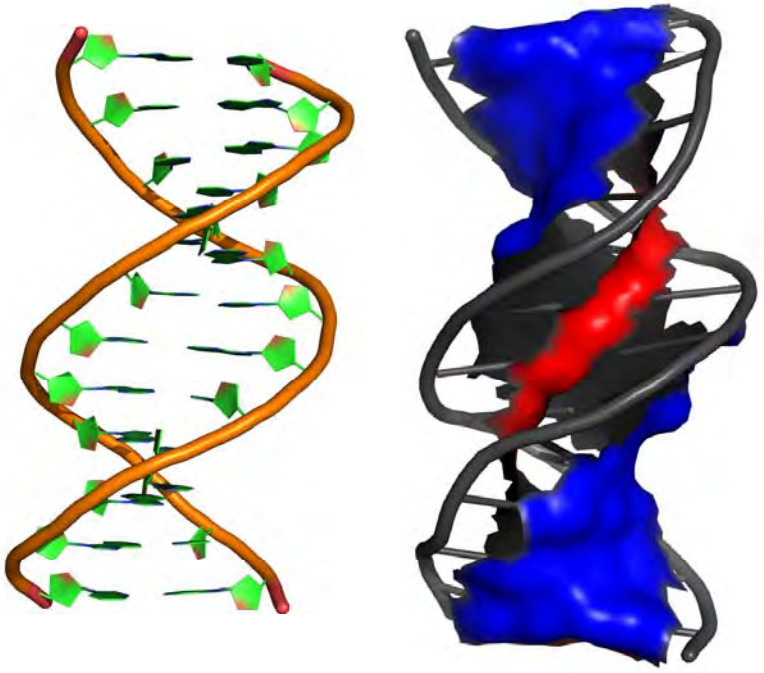
- Biopolymer used for information storage
- Present in a majority of cells in the body
- Made up of repeating base pairs:



Adenine (A) Thymine (T)



Guanine (G) Cytosine (C)

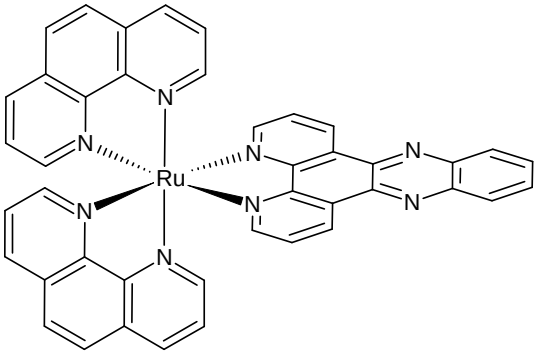


B-DNA – **425D**

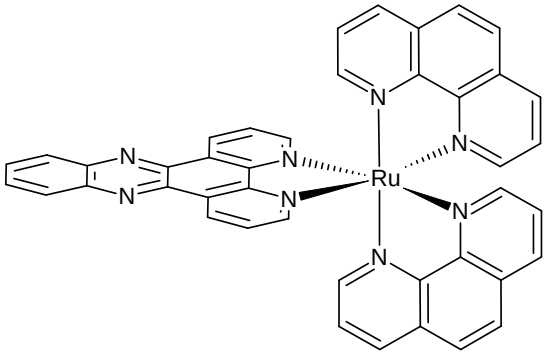
R = deoxyribose sugar – phosphate backbone

Ruthenium Complexes

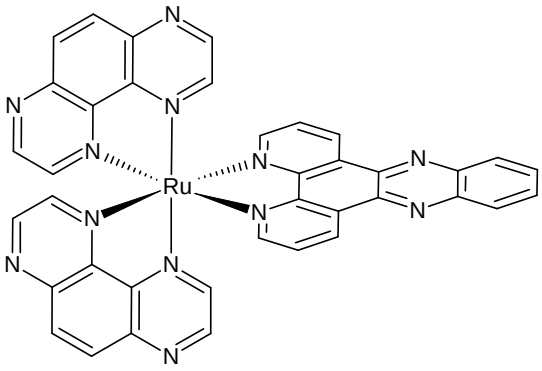
- Polypyridyl ruthenium complexes are known to bind to DNA, can have a fluorescence “light-switch” effect and can induce DNA damage having an anticancer effect[1]
- However, previous work was either spectroscopic or hydrodynamic and there has been no definitive structural evidence.



Λ-$[Ru(phen)_2dppz)]^{2+}$



Δ-$[Ru(phen)_2dppz)]^{2+}$



Λ-$[Ru(TAP)_2dppz)]^{2+}$

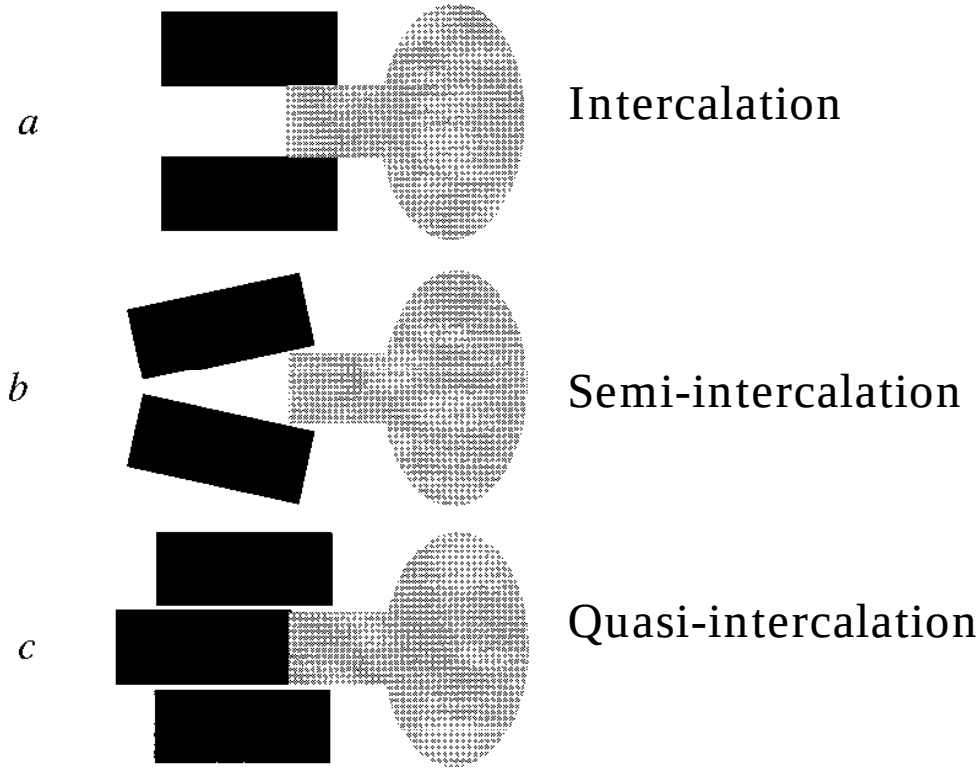
1. Yadav, A. et al. (2013) *Mol Cancer Ther*. **12**, 643-653.

Previous Literature

- Conflicted
- Papers detailing every possible binding mode
- This is because of a lack of structural information

Binding modes

- Three binding modes predicted for these complexes



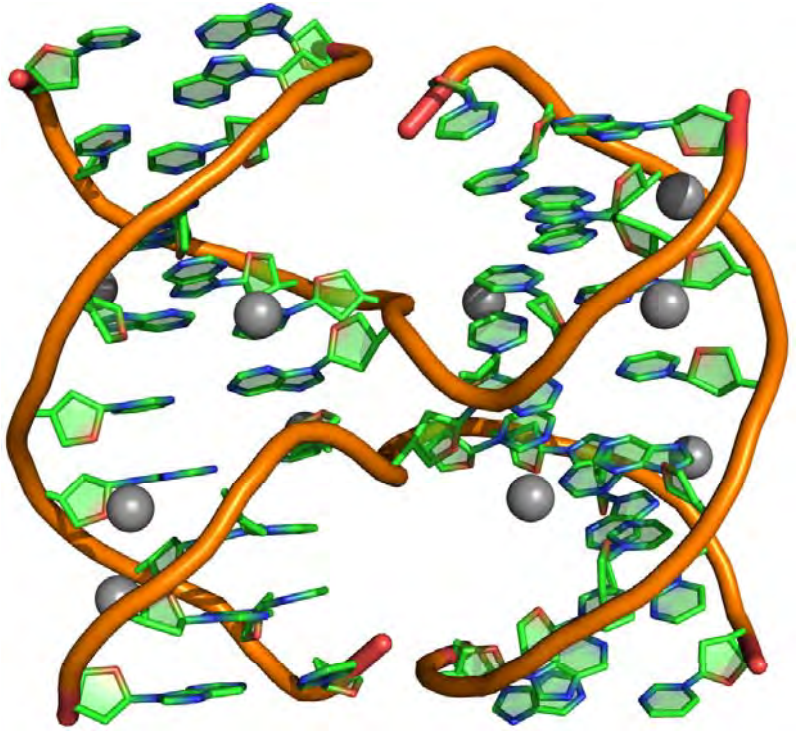
Lincoln, P. & Nordén, B. (1998). *J. Phys. Chem. B.*, **102**, 9583-9594.

Initial Crystallization Trials

- Used the Hampton Research Nucleic Acid Mini Screen (24 unique conditions)
- The sequence d(TCGGCGCCGA) was used with Λ-[$Ru(TAP)_2(dppz)$].2Cl
- Hits were obtained in all barium containing drops
- No hits were seen with other cations in the absence of Ba^{2+} e.g. Sr^{2+}, K^{+}, Na^{+}

Junction Structure

- The sequence d(TCGGCGCCGA) was previously crystallized in the presence of barium only
- This gave the Holliday junction form, with barium cations bound in the DNA grooves

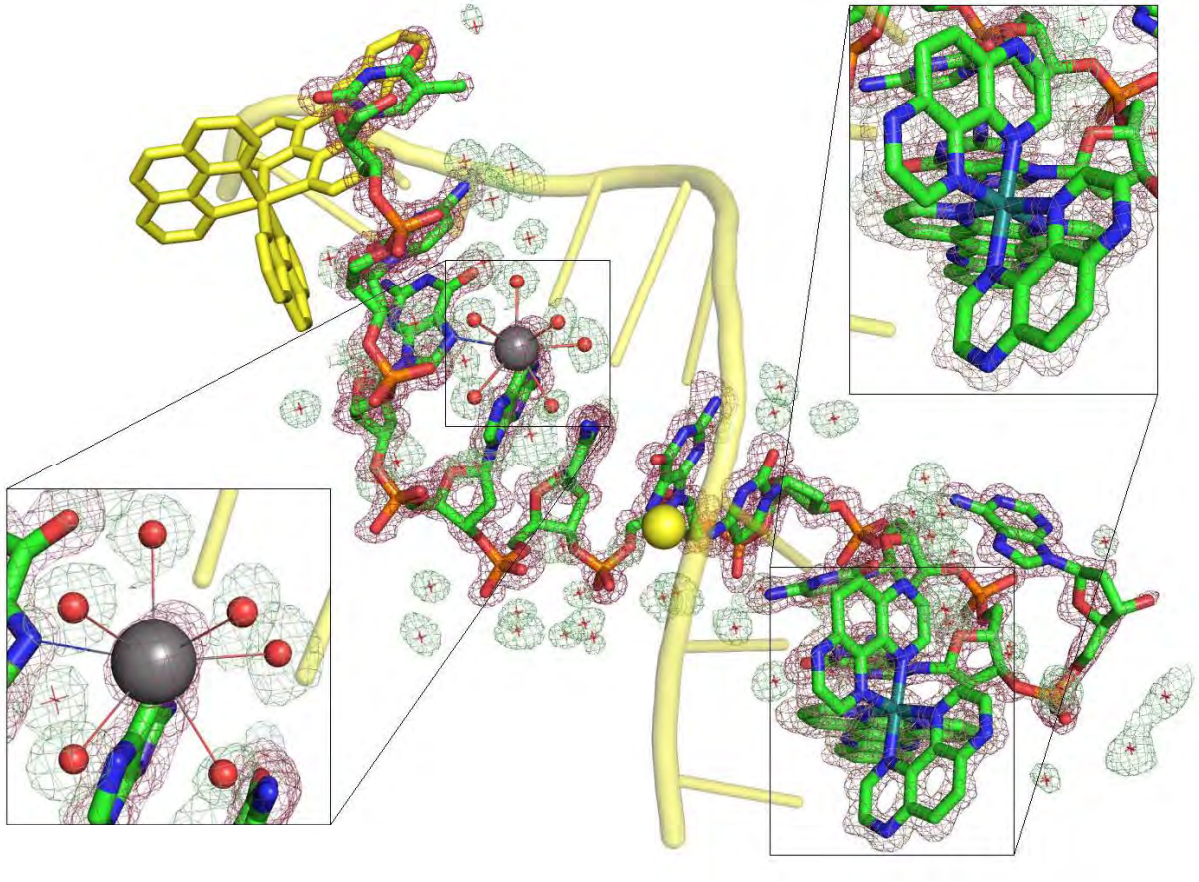


3GOM

Hall, J. P., O'Sullivan, K., Naseer, A., Smith, J., Kelly, J. M., Cardin, C. J. (2011). *Proc. Natl. Acad. Sci.* **108** 17610-17614.

Duplex Structure

- d(TCGGCGCCGA) + Λ-Ru(TAP)$_2$dppz
- Crystals were grown using the complex and DNA in the presence of barium chloride, spermine and cacodylate buffer at 277 K.



Data Collection	
Space group	$P\,4_32_12$
Cell dimensions	
a, b, c	42.3, 42.3, 39.9
Resolution, Å	21.16-1.10 (1.16-1.10)
R_{merge}	0.066 (0.452)
I/σI	24.6 (6.1)
Completeness, %	99.6 (100)
Multiplicity	15.8 (16)
Refinement	
Resolution	18.93-1.10
No. Reflections	13340
R_{work}/R_{free}	0.108/0.124
No. Atoms	
DNA	202
Ligand	51
Water	64
Average B factors	
DNA	14.8
Ligand	11.96
Water	24.03
Rmsd	
Bond lengths	0.0273
Bond angles	3.044
PDB ID	**3QRN**

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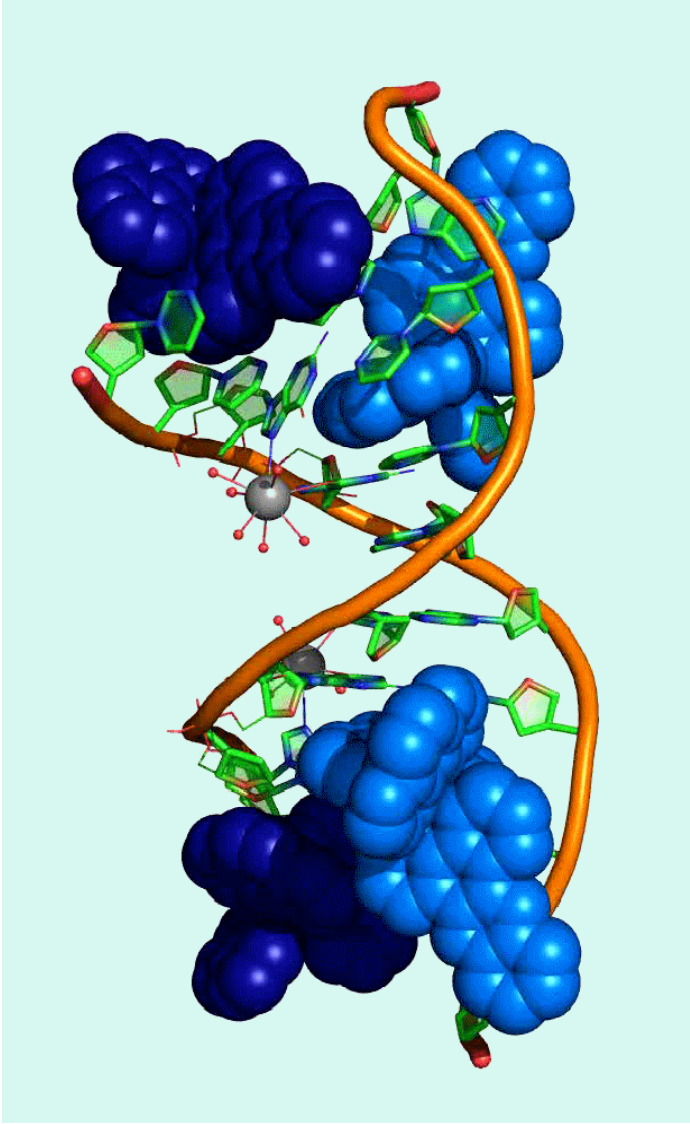
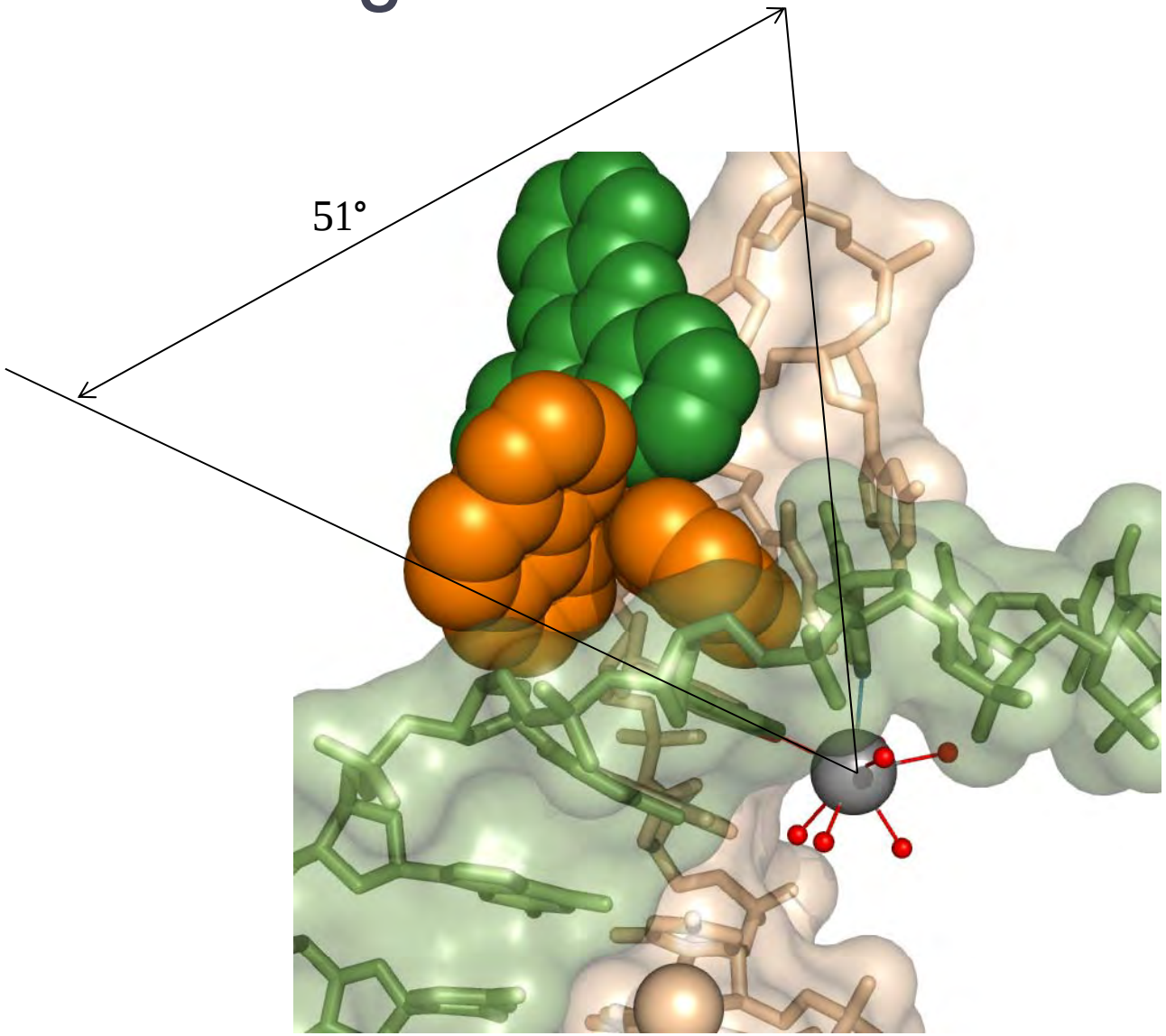
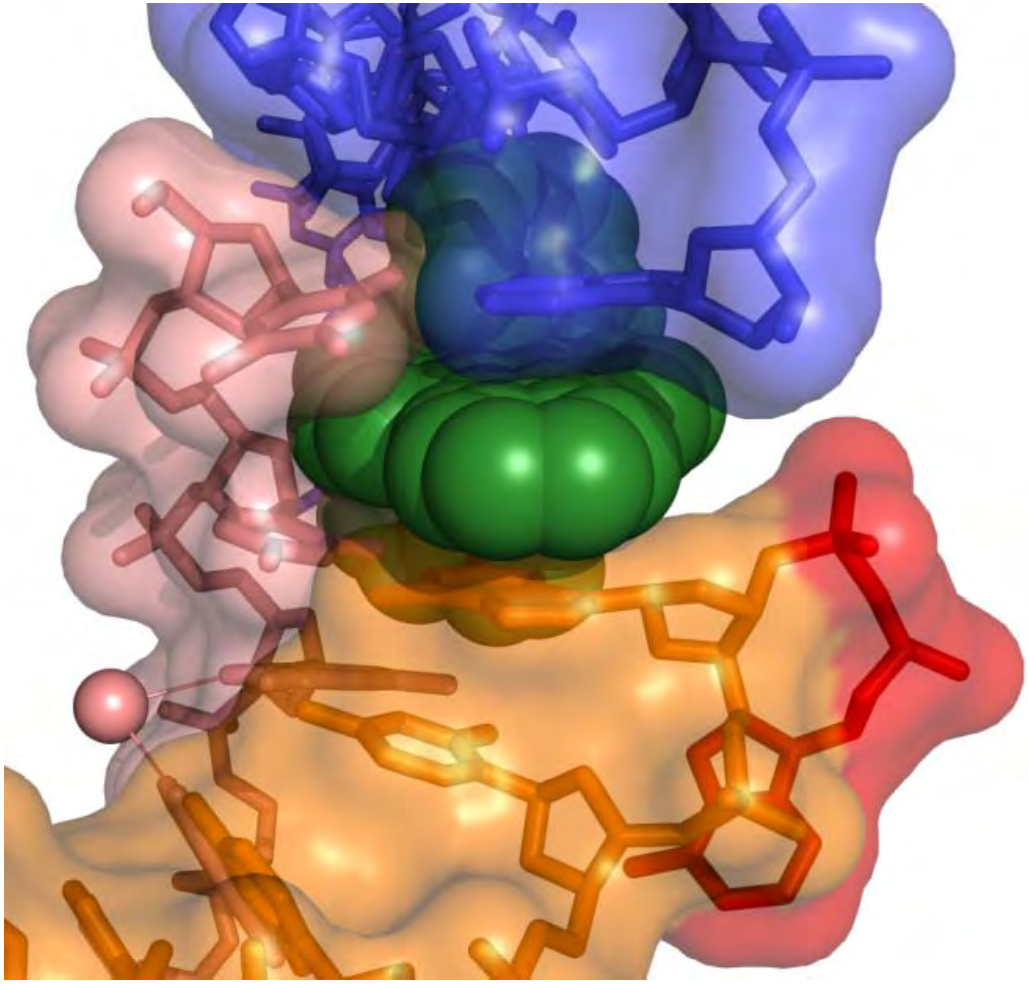
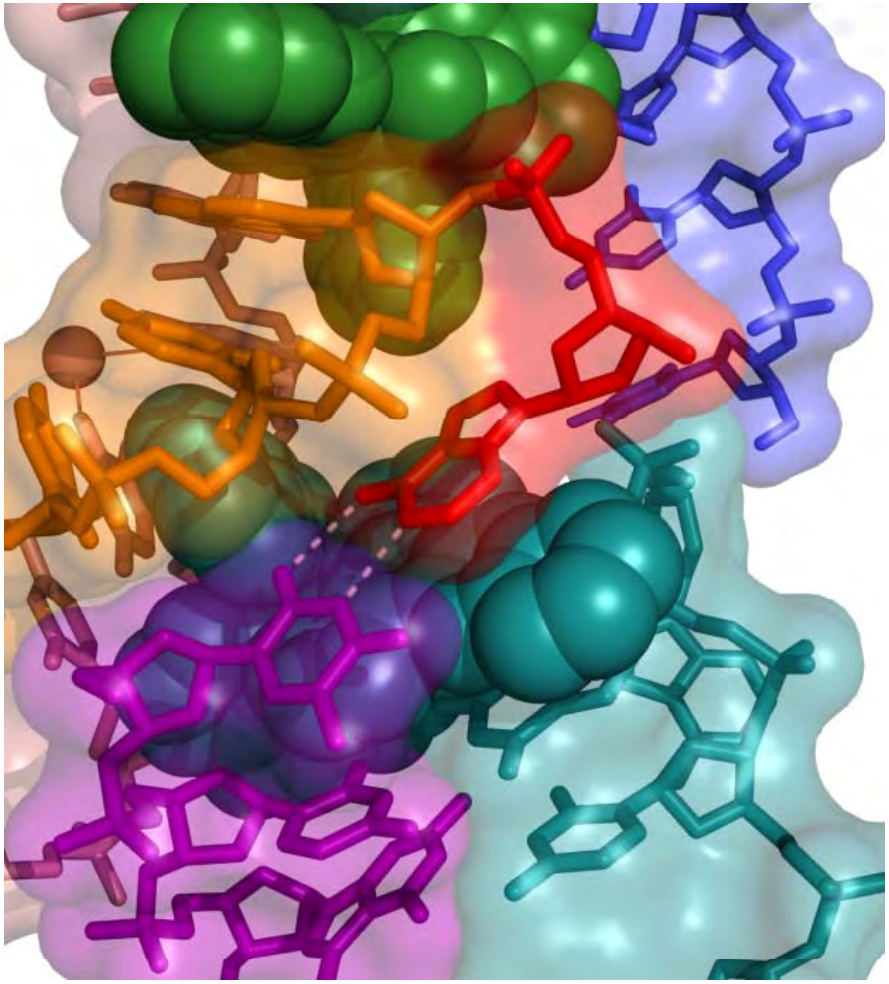
Duplex Structure



Kinking



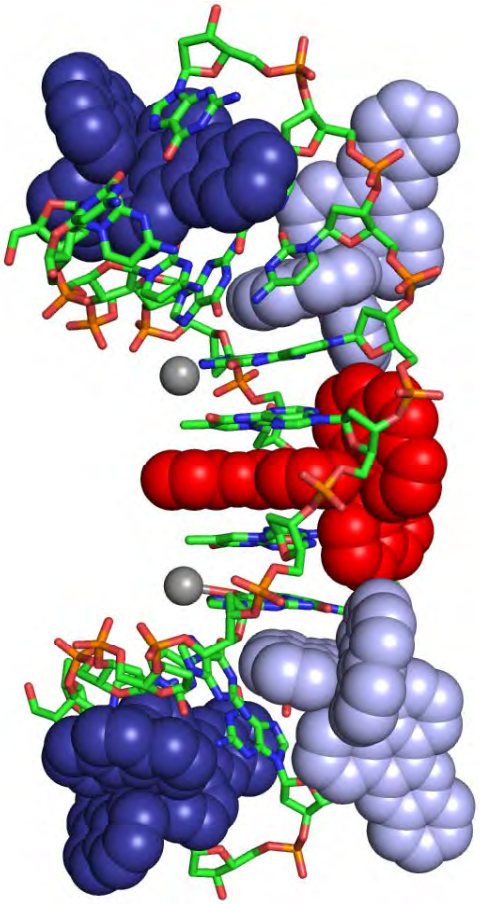




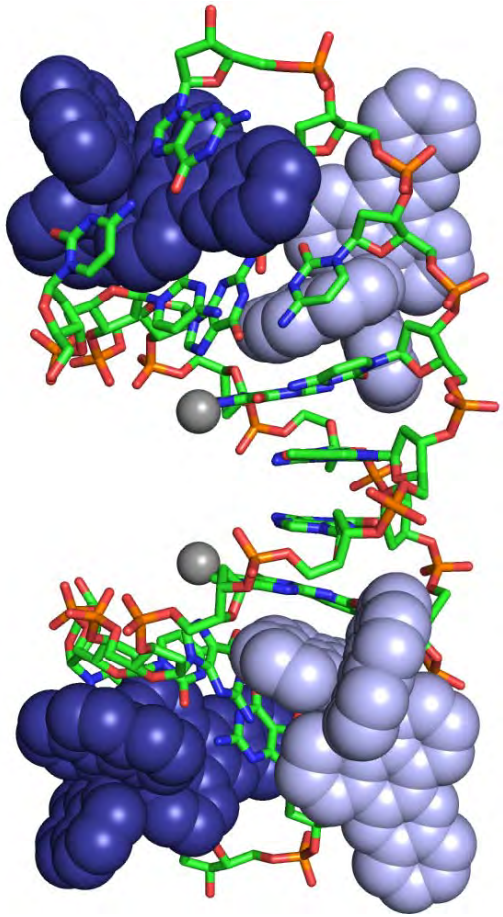
Sequence Specificity

- Next we tried to crystallize two sequences d(CCGGTACCGG) and d(CCGGATCCGG)
- The crystallization conditions were similar to those listed previously
- This was with racemic $[Ru(phen)_2(dppz)]^{2+}$

Sequence Specificity



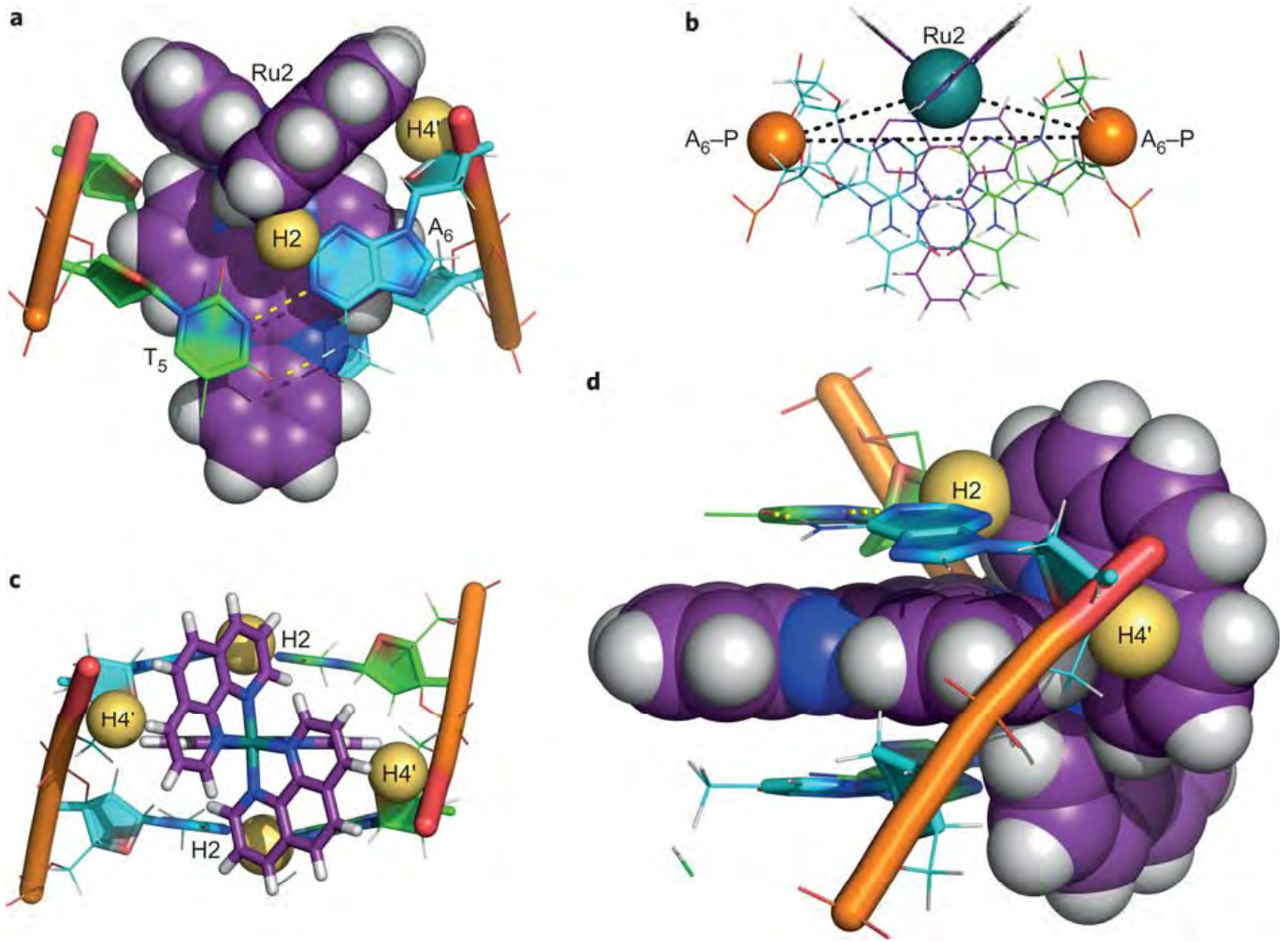
d(CCGGTACCGG)$_2$
3U38



d(CCGGATCCGG)$_2$
4E7Y

Niyazi, H., Hall, J. P., O'Sullivan, K., Winter, G., Sorensen, T., Kelly, J. M., Cardin, C. J. (2012). *Nat. Chem.* **4**, 621-628.

Sequence Specificity



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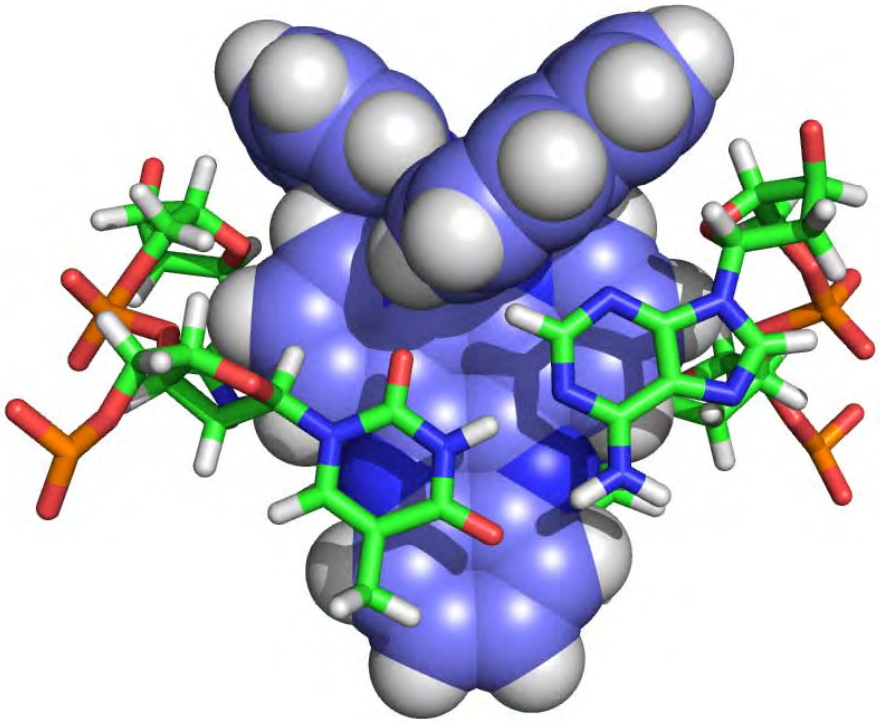
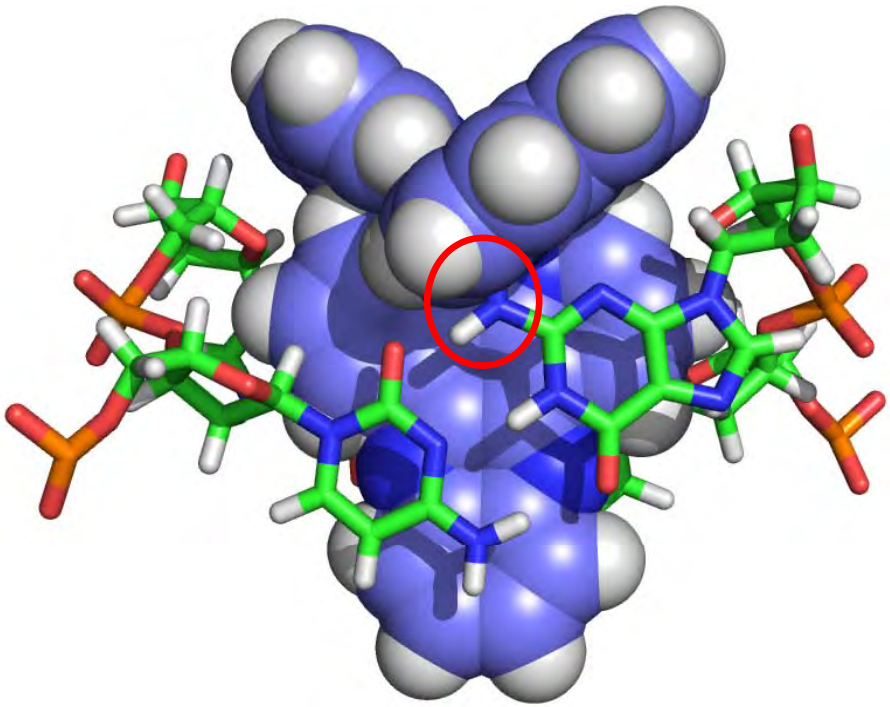
Sequence Specificity



Base Pair: T-A

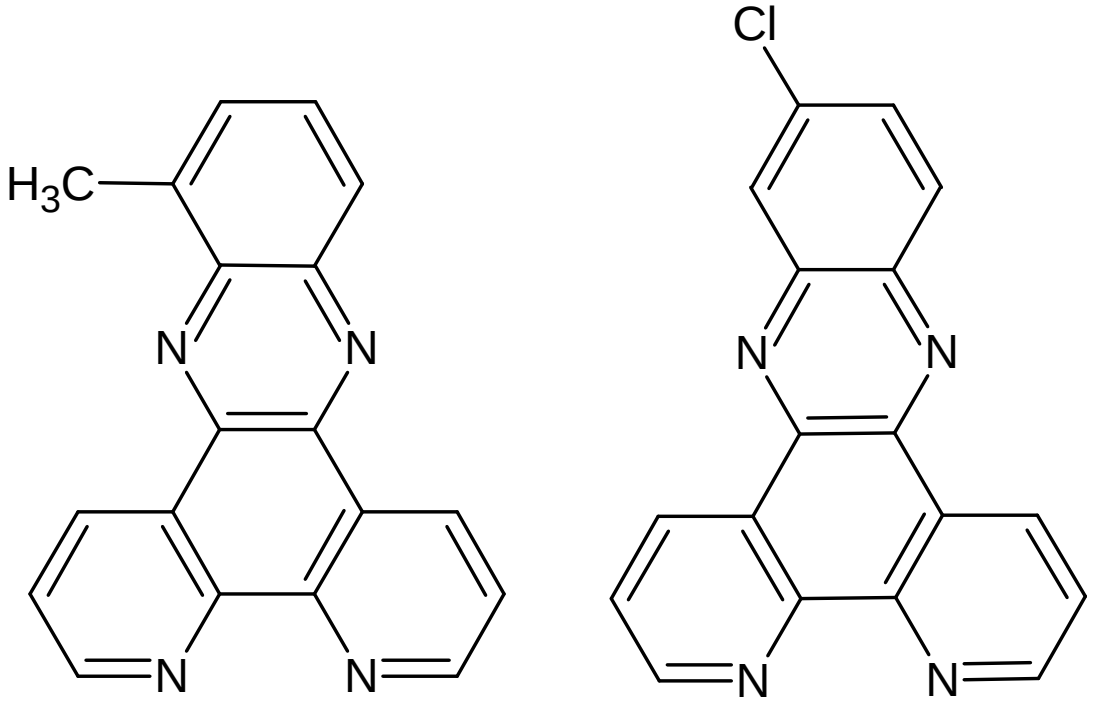


Base Pair: C-G (Mutated from TA model)

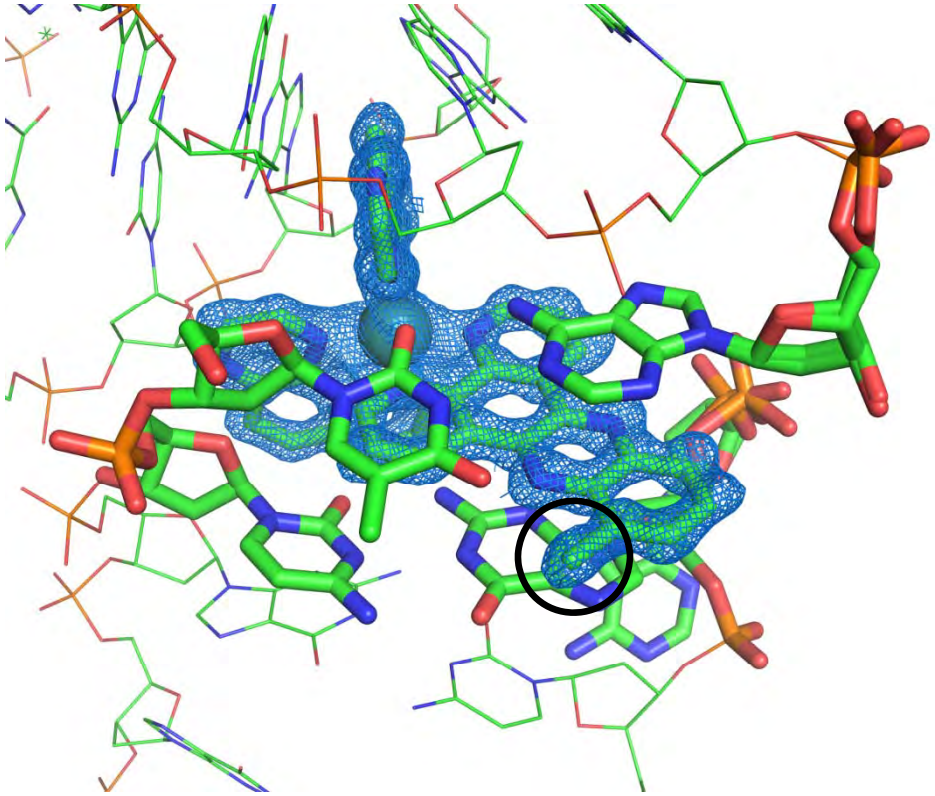
Niyazi, H., Hall, J. P., O'Sullivan, K., Winter, G., Sorensen, T., Kelly, J. M., Cardin, C. J. (2012). *Nat. Chem.* **4**, 621-628.

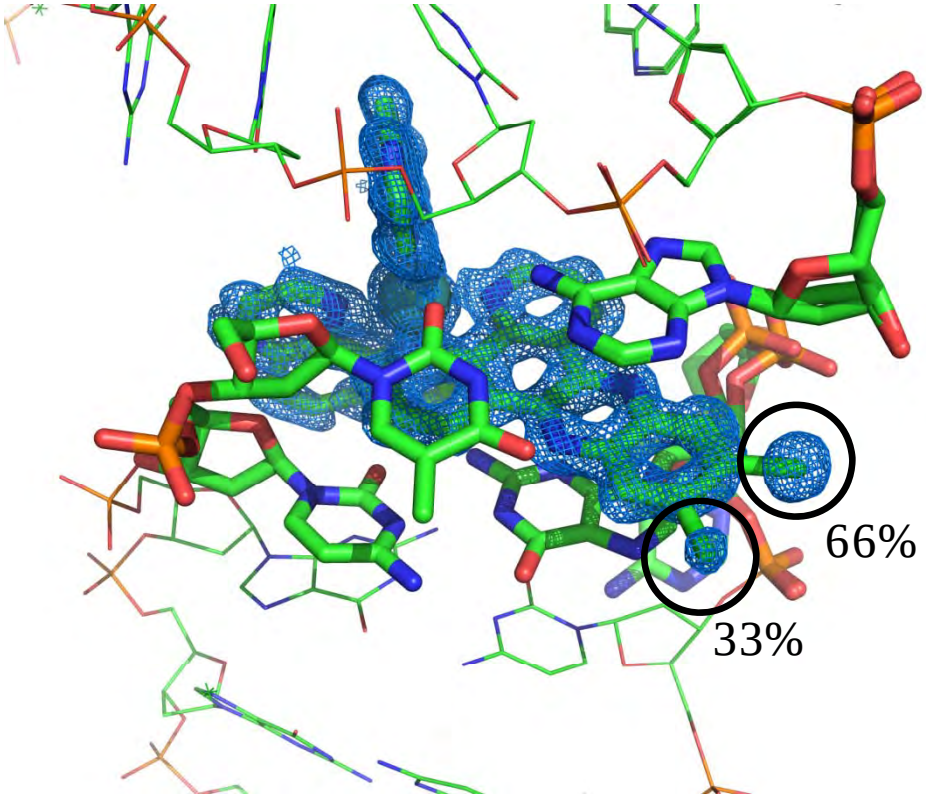
Directional Preference

- The next step was to introduce small changes to the $[Ru(TAP)_2(dppz)]^{2+}$
- 10-Me and 11-Cl derivatives were produced
- Both crystallized with $d(TCGGCGCCGA)_2$ and gave atomic resolution data



Directional Preference





• 10-Me binds with the methyl pointing into the major groove

Resolution: 0.96 Å

• 11-Cl is disordered over two sites.

4III
Resolution: 1.02 Å

There is no steric reason for this in the structure. The difference could be electronic.

Hall, J. P., Beer, H., Buchner, K., Cardin, D. J. & Cardin, C. J. (2013) *Philos. T. Roy. Soc. A.* **371**, 20120525.

Conclusions

- Both semi-intercalation and intercalation occur
- Ruthenium complexes bind with a high level of sequence specificity, preferring TA sites over AT and CG
- Positions of hydrogen atoms could determine the binding specificity
- Introducing small changes into the complexes can change the orientation at the binding site

Future Work

- Crystallize a wide range of sequences to try and observe binding, or lack of, for every possible DNA step
- Perform solution measurements on short oligonucleotide sequences
- Scope for neutron diffraction experiments to obtain actual hydrogen positions
- Investigate the photochemistry in the crystal to compare to that in solution

Acknowledgements

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