Crystallization and structural characterization of triplexes and triplex-ligand systems using DNA X-ray crystallography

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Introduction

- The DNA triplex can be formed by the introduction of a triplex-forming oligonucleotide (TFO) to a DNA duplex.[1] (Fig. 1). A full understanding of triplex structure and chemical modification strategies is essential.
- Here we show results describing the behaviour of triple helical molecules in UV-visible and circular dichroism spectroscopy.
- Additionally, we present the DNA structure obtained with X-Ray crystallography of a dsDNA belonging to a studied triplex DNA system.

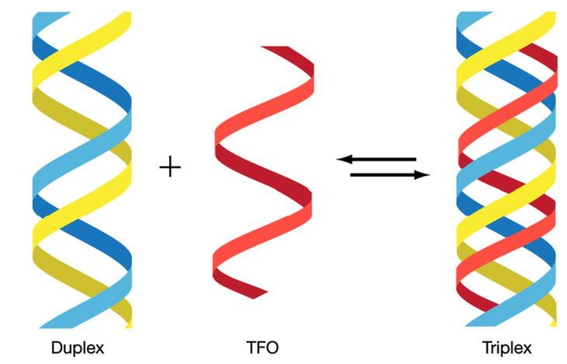

Figure1 Schematic representation of intermolecular DNA triplex formation. A TFO, indicated in red, will bind the target duplex through the major groove.[1]

Results and Discussion

The TFO can be an independent strand (intermolecular) or from the same DNA strand (intramolecular).[2] A set of intermolecular triplexes and intramolecular triplexes have been selected for investigation as model systems, representing broad coverage of the sequence diversity observed for triplex systems.

Absorbance Melting Profile

- The identified systems were examined using UV-visible absorption spectroscopy, to identify conditions in which they displayed the highest thermal stability.
- Conditions tested include pH, salinity, concentrations of precipitants and how the presence of other ligands, such as Ethidium bromide (EtBr), affected the stability of the triplex assembly.
- Here we show a melting curve of an intramolecular triplex with two transitions (Fig. 2A), a comparison duplex and a triplex melting curve of an intermolecular triplex DNA (Fig 2B) and how EtBr, is used as a stabiliser, showing a second transition, although unstable. (Fig. 2C)

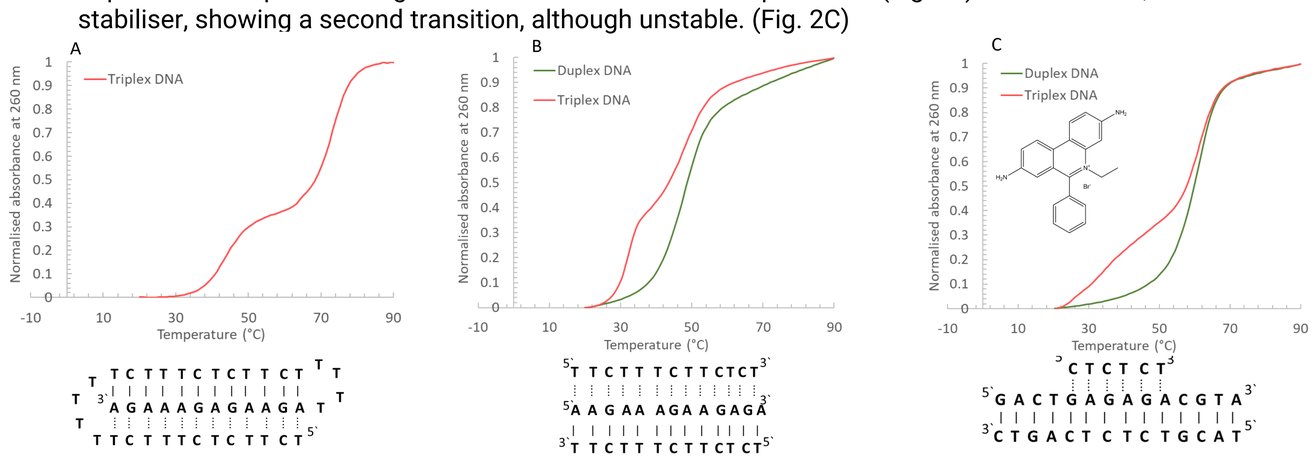

Figure 2 Intramolecular triplex DNA normalized UV melting curves at pH 7.0, 100 mM of NaCl and 10 mM $MgCl_2$. (A) Comparison of duplex DNA and intermolecular triplex DNA normalized UV melting curves at pH 7.0, 100 mM of NaCl and 10 mM $MgCl_2$. (B) Comparison of duplex DNA and triplex DNA normalized UV melting curves at pH 5.5, 100 mM of NaCl , 10 mM $MgCl_2$ and 2uM of EtBr. (C)

Circular Dichroism Analysis

- The structures of the DNA triplexes were evaluated by circular dichroism (CD) spectroscopy.
- Hoogsteen pairs are less stable and dissociate at lower temperatures than the strands with Watson-Crick pairs.[3]
- The band is negative at 210-220 nm for temperatures below 30°C (dashed, red). This confirms the formation of triple helical structures and the loss of Hoogsteen-paired strand when the temperature is increased (solid lines, grey shades).

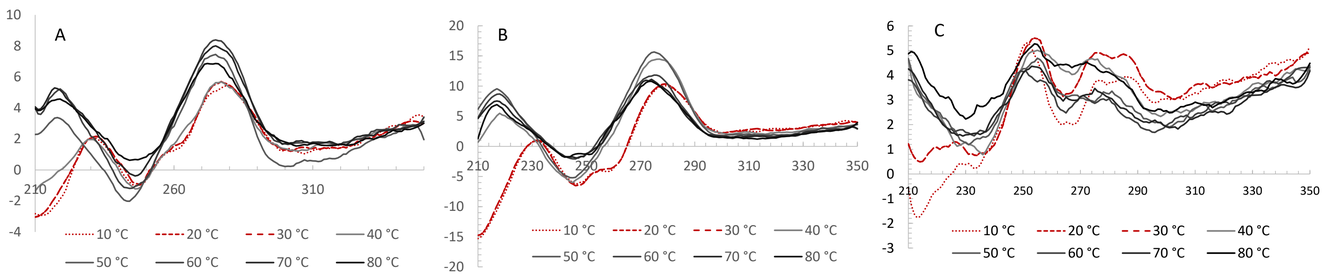

Figure 2 Measured CD spectra from 10-80 ℃ with 10 ℃ increment. For Intramolecular DNA triplex (A) and intermolecular DNA triplex (B-C)

Future Perspective

- The aim of this project is to explore the structural properties of DNA triplexes using X-ray crystallography
- The results obtained are a promising starting point to proceed with further optimizations.
- As the TFO will bind to the duplex with sequence specificity, there is significant interest in developing TFOs with potential therapeutic applications.
- Since intercalators are studied for functionalising DNA triplexes, Ru(II) polypyridyl compounds have been investigated to exploit their photophysical, electronic and biological properties.

Crystallization

Crystallization screening testing several chemical and environmental conditions were undertaken, followed by optimization steps to improve size or morphology. (Fig. 4 A-F)

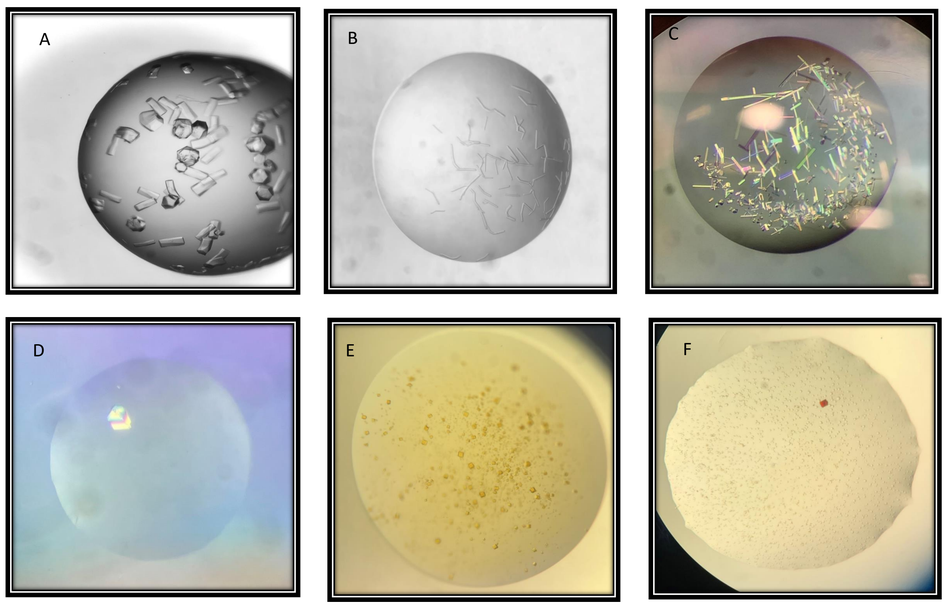

Figure 4 Crystals obtained in plates prepared with DNA triplexes. (A-F) Crystals prepared with Ru(II) polypyridyl complexes. (E-F)

Crystal Structure of a non-canonical triplex

- Crystal structure of dsDNA obtained from a DNA triplex crystallization attempt.
- The NMR structure, deposited in the PDB, shows the presence of a TFO. However, here the duplex DNA crystallised on its own.
- Interestingly, we observed a base flipping out and binding in the minor groove of the symmetry-related molecule.

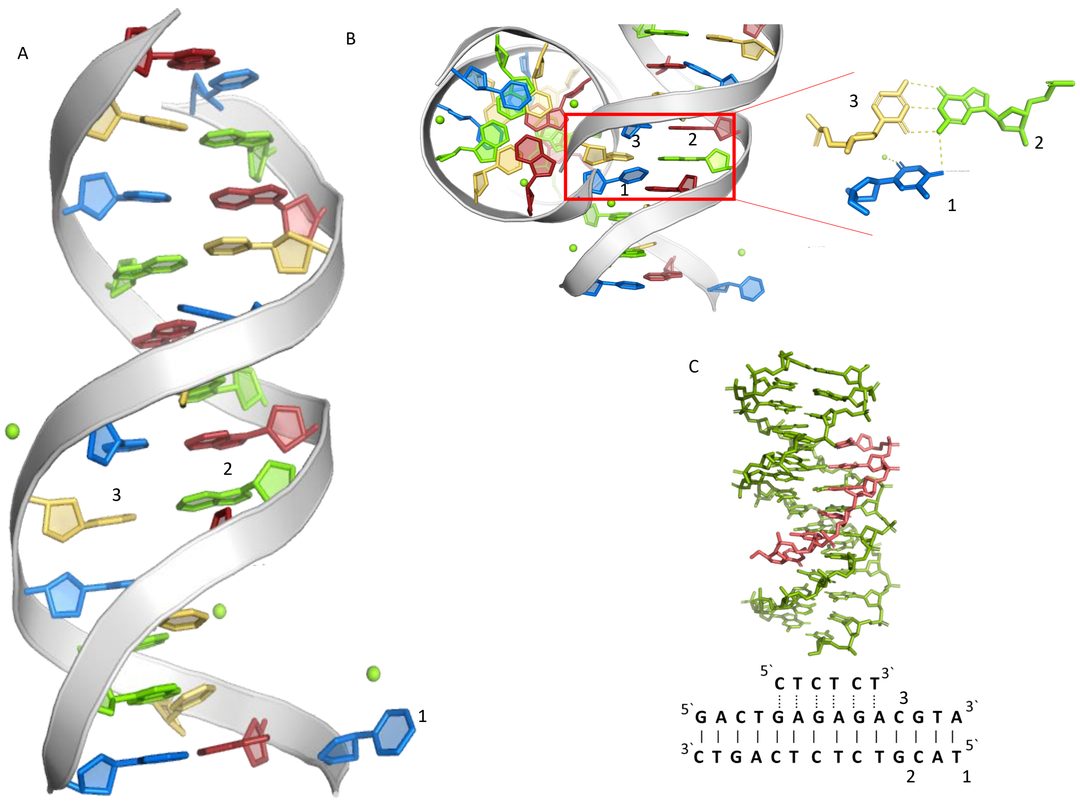

Figure 5 Crystal structure of a dsDNA. (A) Section of DNA highlighting the flipping base in the minor groove. (B) NMR structure of triplex DNA PDB:1BWG (C)

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