Peptides Targeting DNA Four-Way Junctions

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https://doi.org/10.17952/37EPS.2024.P1018

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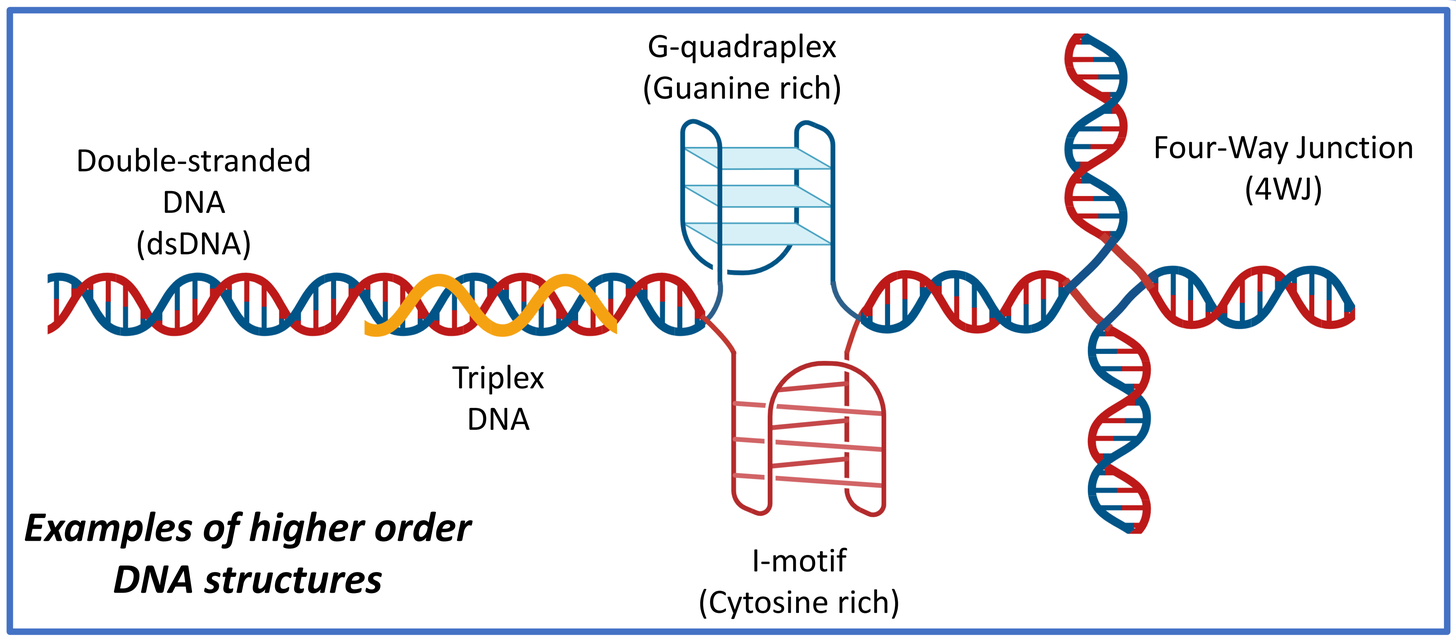
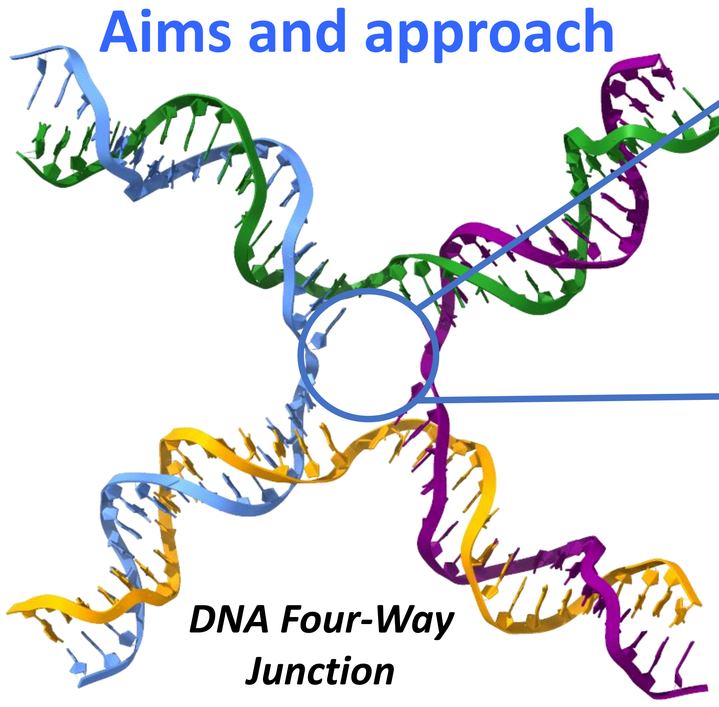
Introduction

- Higher-order DNA structures are formed by the assembly of DNA into various 3D shapes.
- Four-Way Junctions (4WJs) are formed by the crossing over of DNA strands belonging to two separate double-stranded DNA segments.
- 4WJs are common intermediates in DNA repair and can be targeted in cancer and bacterial cells.[1]

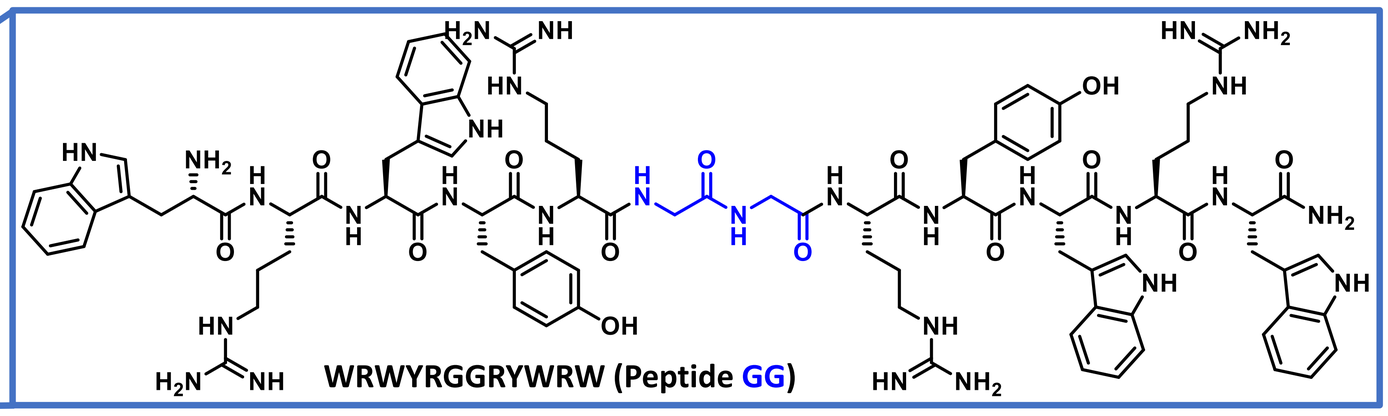


Examples of higher order DNA structures

Aims and approach



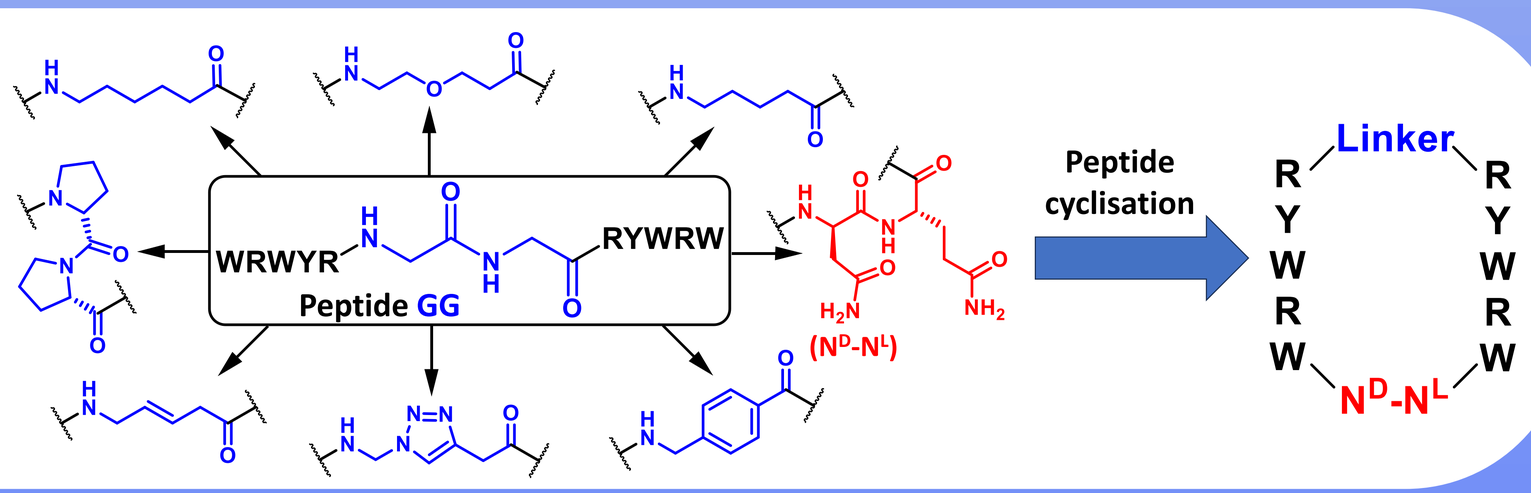
DNA Four-Way Junction



- Peptide GG binds to the DNA 4WJ and has anti-bacterial effects.[2]
- During this project, peptides analogues were synthesised, based on the structure of Peptide GG.
- A series of biological assays were developed to test the ability of the peptide analogues to bind to the DNA 4WJ.

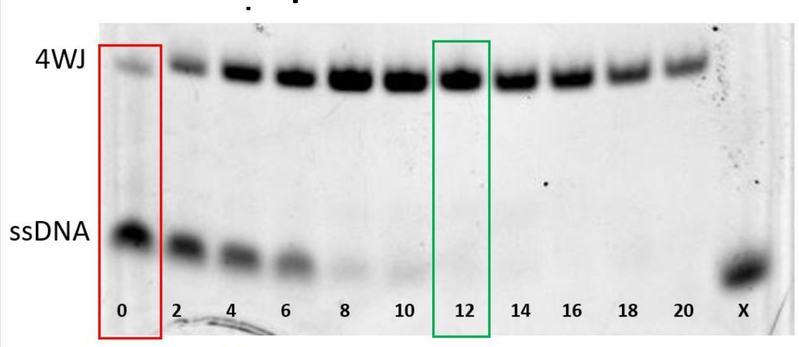
Results - synthesis

- Peptides synthesised using Solid-Phase Peptide Synthesis (SPPS).
- GG replacement groups (linkers) were added as unnatural amino acids.
- Cyclic peptides were produced, by combining the N^D-N^L linker with another linker.



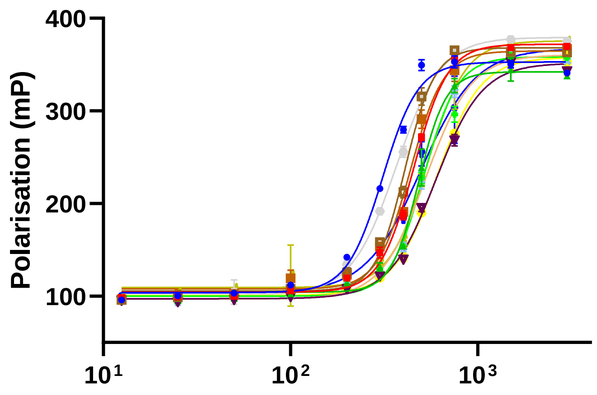
Results – Biological

Gel electrophoresis[3]



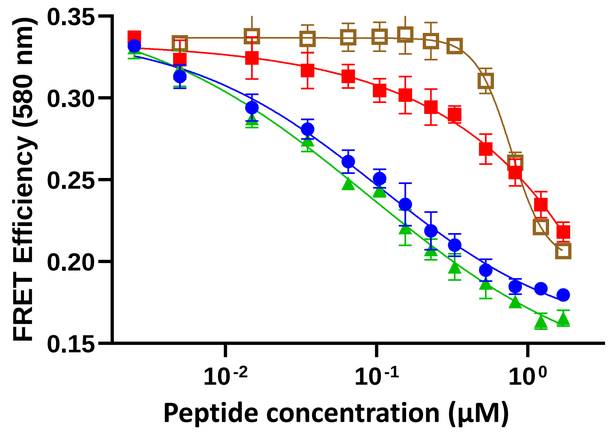
When four ssDNA strands were annealed in the absence of peptide, mostly ssDNA was formed. In the presence of peptide GG, the DNA was fully trapped as 4WJ.

Fluorescence Polarisation[4,5]



An increase in polarisation was observed when peptide was titrated into Fam-labelled 4WJ DNA.

Förster Resonance Energy Transfer (FRET)[6]



In solution the 4WJ was X-stacked, allowing FRET to occur. As peptide was added the 4WJ opened and FRET decreases.

Similar results were obtained for the linear/cyclic peptide analogues.

Different rates of 4WJ opening were observed. This indicates that although the peptides may have similar binding affinity, they may have different binding modes.

Future work

- Test the peptide analogues for selectivity against other DNA structures.
- Screen the peptide analogues for antibacterial activity, comparing to Peptide GG.
- Obtain crystal structures of the peptide:4WJ complex for further SAR studies.

References: 1) Ivens et al, *Bioorg. Med. Chem.* **2022**, 69, 116897. 2) Rideout et al, *Peptides.* **2013**, 40, 112. 3) Howell et al, ChemMedChem. 2012, 7, 792. 4) Fogg et al, J Mol Biol. 2001, 313(4), 751. 5) Lin et al, Int. J. Mol. Sci. 2023, 24(1), 580. 6) Clegg et al. Biochemistry, 1992, 31(20), 4846.