X-ray Crystallographic and Photophysical Studies of DNA i-motifs

Sarah P. Gurung[a,b,c], James P. Hall[a,b,c], Graeme Winter[b], John A. Brazier[d], Thomas Sorensen[b,c] and Christine J. Cardin[a,c]

[a]Department of Chemistry, University of Reading, Reading, UK; [b]Diamond Light Source, Didcot, UK; [c]The Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot, UK; [d]School of Pharmacy, University of Reading, Reading, UK

An i-motif is a four stranded structure made of cytosine-rich DNA sequences. Its sequence is usually in the format of $C_{2\text{-}5}L_{1\text{-}9}C_{2\text{-}5}L_{1\text{-}9}C_{2\text{-}5}L_{1\text{-}9}C_{2\text{-}5}$, where C is cytosine and L represents any other base. The conformational change from the C-rich single strand DNA to i-motif takes place between pH 5 and 6.7. These acidic conditions help two parallel i-motif duplexes "zip" together in an antiparallel orientation by protonating N3 in cytosines to create hemiprotonated C–C^+ base pairs (fig. 1).[1]

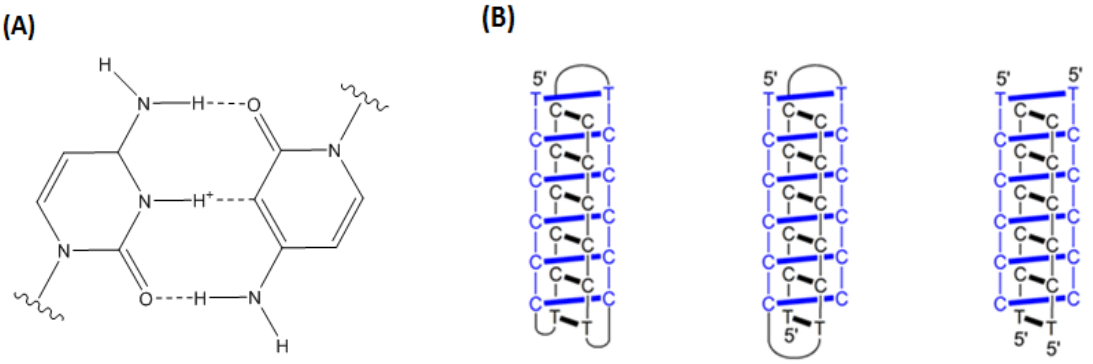

Figure 1. (A) C-C^+ base pairing in i-motifs. (B) Schematic diagrams of unimolecular (left) bimolecular (middle) and tetramolecular (right) i-motifs.

The i-motif can form as either an inter- or an intramolecular structure. However, only six i-motif crystal structures have been reported on the NDB; all of which are tetramolecular, even though i-motifs *in vivo* would exist as unimolecular. The c-Myc, Bcl-2 and hTERT i-motifs are all unimolecular and are present in the promoter regions of their respective oncogenes.[2] Like the guanine rich G-quadruplex, the presence of a cytosine rich sequence has also been detected within the promoter region of the human telomeric and centromeric DNA, making i-motifs an attractive subject for gene transcription modulation.

UV and synchrotron radiation CD (srCD; beamline B23 at Diamond Light Source) spectroscopy were used to study the structural stability of intramolecular i-motifs. Our results showed that i-motifs with shorter loop lengths exhibit the highest stability.[3] Crystallisation trials based on these initial results will be discussed along with previously recorded i-motif crystals grown in new conditions. We will also be reporting the diffraction of $d(CCCT)_4$ crystals at 0.68 Å at beamline I02, illustrating the advances in modern-day DNA crystallography via synchrotron radiation. Combination of results from the mentioned instrumental approaches shows that these methods are actually complementary.

1. Gehring, K., Leroy, J. L. & Gueron, M. A tetrameric DNA structure with protonated cytosine.cytosine base pairs. *Nature,* **1993**. 363**,** 561–565.
2. Phan, A. T. & Mergny, J.-L. Human telomeric DNA: G-quadruplex, i-motif and Watson-Crick double helix. *Nucleic acids research,* **2002**. 30, 4618–25.
3. Gurung, S. P., Schwarz, C., Hall, J. P., Cardin C. J. & Brazier, J. A. The importance of loop length in the stability of i-motif structures. *Chem. Commun.* **2015**. 51, 5630-32.