NMR studies of tetrahelical DNA structures and their ligand interactions

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DNA with its canonical double helix plays an important role in the inheritance of genetic material and gene expression. However, various methods, including NMR, show that DNA oligonucleotides are increasingly more polymorphic than commonly thought and are far from being associated only with the double helix described in the early 1950s. Multiple strands can assemble into complex higher-order DNA structures, such as four-stranded G-quadruplexes (G4) adopted by guanine-rich regions. Different DNA structures open up multiple possibilities for targeting due to their local structural and dynamic features.

GGGAGCG repeats occur in the regulatory regions of genes responsible for neurological disorders, cancer, and abnormalities in bone and cartilage development. These G-rich fragments form unique tetrahelical structures that are distinctly different from G-quadruplexes. The core of the structure is stabilized by the formation of GAGA-quartets, major and minor groove GCGC-quartets, and exhibits G-A base pairs stacked on G-G base pairs that from the loop regions of three Gs (pdb id: 2MJJ, *Nat. Commun.* **2017**, *8*:15355). Interestingly, similar to B-DNA, the AGCGA-quadruplex is stabilized by hydrophobic desolvation and, unlike G-quadruplexes, also by specific binding of water molecules (*Angew. Chem. Int. Ed.* **2019**, *58*, 2387). Their respective complexes with bis-quinolinium ligand 360A, reported to have high affinity for G-quadruplexes and to selectively inhibit telomerase, were found to intercalate between GAGA- and GCGC-quartets in the central cavity of AGCGA-quadruplex (*Chem. Eur. J.* **2020**, *26*, 814).

Human telomeric G-quadruplex DNA structures are attractive targets for anticancer drug development, but polymorphism of the target complicates their design. Different ligands prefer different folds, and very few complexes have been solved at high resolution. Phen-DC_3, bisquinolinium-derivatized phenanthroline-dicarboxamide, one of the best-known G-quadruplex ligands characterised by high binding affinity and selectivity, causes dTAGGG$(TTAGGG)_3$ to completely change its fold in KCl solution from a hybrid-1 to an antiparallel chair-type structure, with the ligand intercalating between a two-quartet unit and a pseudo-quartet, ejecting a potassium ion. The unprecedented high-resolution NMR structure is the first to show true ligand intercalation into an intramolecular G-quadruplex (*Angew. Chem. Int. Ed.* **2022**, *61*, e202207384).