Small Molecule Targeting T:T Mismatches in CTG Trinucleotide Repeat DNA Induces a Unique Structure

The current study describes new structural features of higher-order non-canonical DNA conformations induced by a small molecule ligand and its selective supramolecular interactions.

The abnormal expansion of repetitive DNA in a genome results in dysfunctional cellular processes such as replication, repair and recombination that ultimately lead to altered genetic processes. DNA repeat expansion is associated mainly with neurological diseases that might have severe consequences for human health. For example, myotonic dystrophy type 1 (DM1) is associated with the abnormal expansion of cytosine-thymine-guanine (CTG) trinucleotide repeats (TNRs) DNA located on the non-coding region of the myotonic dystrophy protein kinase gene.[1] Expansion of the aberrant TNRs is typically correlated with genetic inheritability, age of disease onset, and severity. Studies have shown that the formation of atypical DNA structures containing mismatches flanked between canonical Watson–Crick base-pairs in the genome is common in disease pathobiology.[2]

As the formation of non-canonical structures has been identified in many neurodegenerative diseases, small molecules targeting these structures can play a significant role in the diagnosis and treatment of these diseases. Triaminotriazine-acridine conjugate (Z1), a drug originally developed by Steven C. Zimmerman (University of Illinois, USA) and his team, that targets mismatches in trinucleotide repeat DNA has the potential to cure DM1 disease (**Fig. 1(a)**),[3] but its binding mechanism remains unclear. A collaborative team led by Ming-Hon Hou (National Chung Hsing University) and Zimmerman used X-ray crystallography and biophysical methods to understand the structural basis for recognition of disease-associated DNA by Z1 (**Figs. 1(b) and 1(c)**). The extraordinarily complex structure solved by Hou and his team showed many unprecedented features that not only throw light on structural details about drug-DNA complexes but also enhance our understanding of the supramolecular chemistry of a chemical compound that causes non-canonical DNA superstructure formation.[4]

The elucidation of a complex crystal structure required the collection of high-resolution X-ray data that was conducted using **TPS 05A** and **TLS 15A1** at NSRRC. The team solved a complex structure of drug Z1 and CTG repeat-associated DNA containing three homopyrimidine T:T mismatches with the SAD method using a brominated oligonucleotide [br5U]TCTGCTGCTGAA. Their crystallographic observations

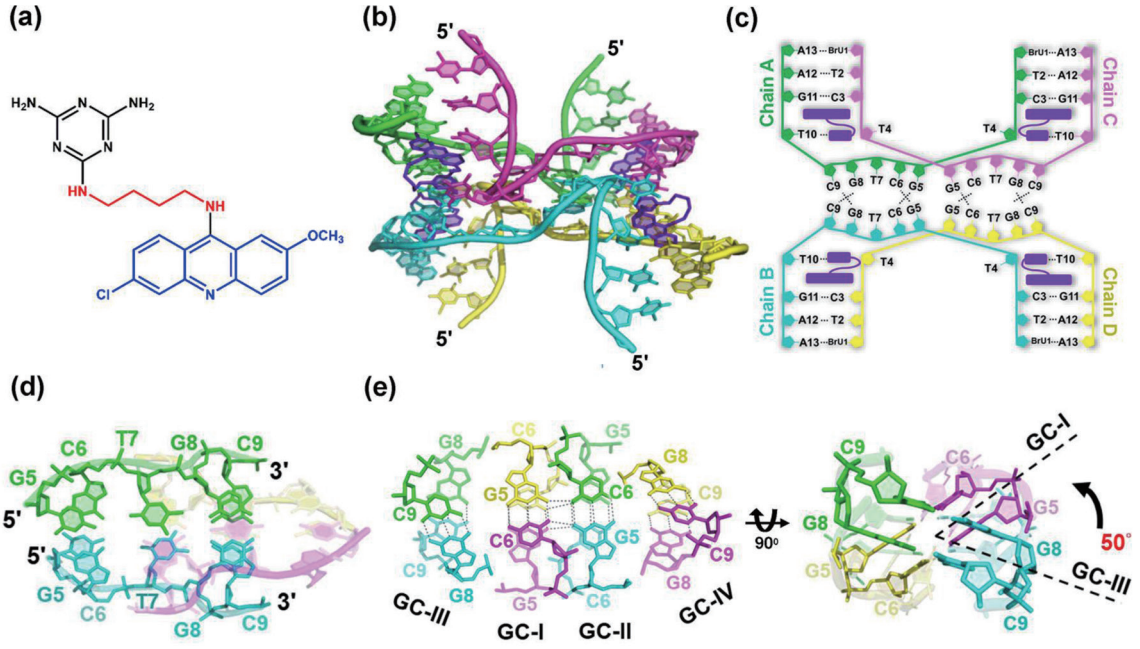

Fig. 1: (a) Chemical structure of triaminotriazine-acridine conjugate (ligand Z1). (b,c) Overall structure and schematic representation of d[(5BrU)T(CTG)$_3$AA] complexed to Z1 in an asymmetric unit. (d) Core of the complex formed by a crossing of all four chains in the G5-C6-T7-G8-C9 penta-sequence. (e) Two central stacked G:C pairings form two GC tetrads labeled GC-I and GC-II. The additional stacked G:C pairings formed by adjacent G8pC9 bases of chains A and C are denoted GC-III and GC-IV, respectively. [Reproduced from Ref. 4]

showed that the binding of two Z1 molecules at the terminal CTG repeat motifs induces many surprising features that include mainly formation of a distorted DNA backbone conformation along with the flipping of a mismatched thymine base away from the backbone that ultimately resulted in a four-way-junction structure of a novel type. The Z1 intercalation caused the four chains of DNA to adopt a double U-shaped head-to-head topology conformation and resulted in the crossing of each strand at the intersection point (**Fig. 1(d)**). Upon further analysis of the structure, Hou's team learned that this central crossing is stabilized with two stacked G:C base pairs in the central region that can rotate by ca. 50° with respect to each other and form an X-shaped structure due to the alignment of two G•C•G•C tetrads into a nonplanar structure (**Fig. 1(e)**). The stacking of G:C pairs on both sides resulted in the formation of an apparently continuous duplex DNA. They further observed that the coordination of four Co^{II} metal ions between N7 atoms of guanines can strongly preserve the central junction site.

As this study identifies the structural basis of a small molecule to induce higher-order structure formation, the information obtained from this work might be applicable in designing more sequence-specific ligands targeting repeat-associated atypical DNA structures, particularly those associated with neurological disorders. In summary, the findings from Hou and his team deepen our understanding regarding the molecular-level mechanism of the formation of higher-order atypical DNA conformations through ligand binding and its supramolecular interactions. Because of the flexibility of DNA to adopt a specific conformation based on a specific sequence or ligand binding, the authors stipulated that the results from this study can be useful also in DNA nanotechnology-based sensors or tweezers development. (Reported by Roshan Satange, National Chung Hsing University)

This report features the work of Ming-Hon Hou, Steven C. Zimmerman and their collaborators published in the J. Am. Chem. Soc. ***142****, 11165 (2020).*

TPS 05A Protein Microcrystallography
TLS 15A1 IASW – Biopharmaceutical Protein Crystallography

- Protein Crystallography
- Biological Macromolecules, Unusual DNA Structures, Life Science

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