organize these structures, and SegB eventually breaks them down to complete the DNA organization process (**Fig. 2(d)**).

The implications of this research extend far beyond basic science. Understanding these fundamental processes could lead to new strategies for controlling bacterial growth, potentially contributing to antibiotic developments. Furthermore, since archaea are considered ancient relatives of complex organisms, these findings provide valuable insights into how DNA organization evolved over time. (Reported by Chun-Hsiang Huang)

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TPS 05A Protein Microcrystallogrphy
TPS 07A Micro-focus Protein Crystallography
TLS 15A1 Biopharmaceuticals Protein Crystallography

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

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Targeting DNA Junctions for Anticancer Drug Development

DNA helix–helix junctions form tetraplex base pairs at the junction interface, serving as "hotspots" for bidirectional bis-intercalating agents. This study investigates the structural basis for targeting DNA junctions with acridine bis-intercalators as a potential anticancer strategy.

Biological processes such as recombination or replication can generate DNA juxtaposed helix–helix structures and duplex crossovers.[1,2] These structures require topoisomerases to decatenate the interlinked DNA crossover sites.[3] Within the crossover structures, the base pairs of the duplexes can interact with each other, resulting in novel junctions. Targeting DNA junction sites with bis-intercalating compounds containing bidirectional linkers could inhibit topoisomerase activity, therefore representing an effective anticancer strategy.[4,5] Bidirectional bis-intercalators have the unique ability to insert their chromophores simultaneously into the base pairs of two DNA duplexes. This non-covalent bridging ability of small molecules enables them to cross-link DNA junctions, thereby disrupting biological processes critical for cellular function. However,

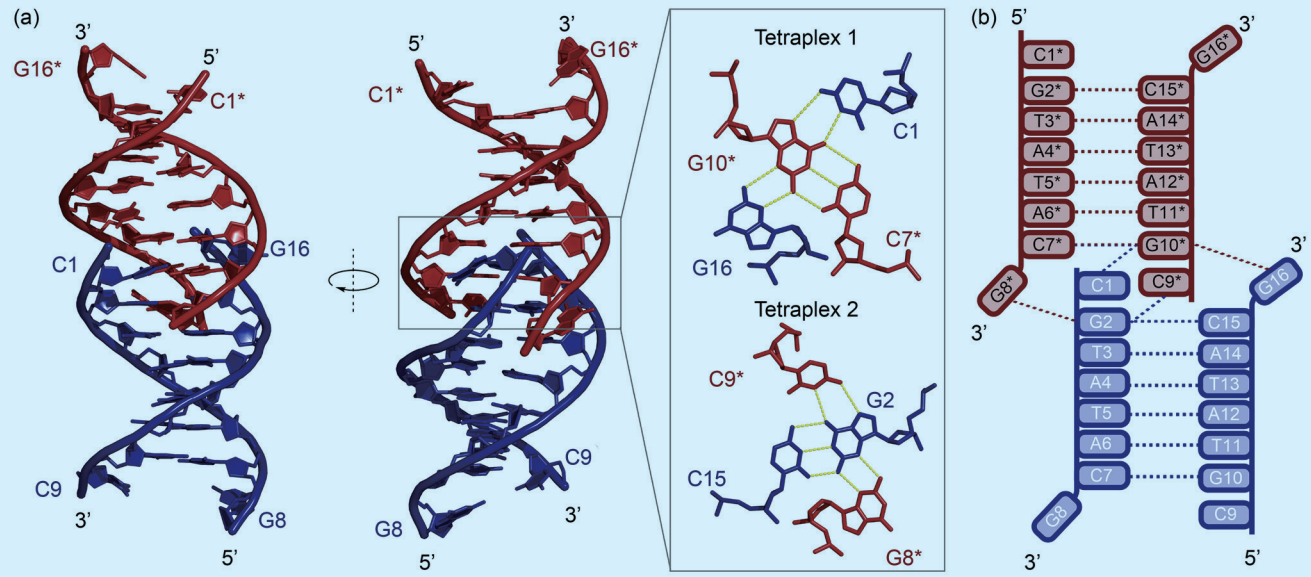

Fig. 1: Structural features of a $d(CGTATACG)_2$ DNA forming junction. (a) Crystal structure assembly of continuous duplexes forming an end-to-end helix–helix junction structure. One DNA duplex is shown in dark blue and the adjacent symmetry-related duplex is in dark red. Asterisks (*) represent residues in the adjacent duplex. Two layered tetraplex base pairings at the junction interface are shown in an enlarged view. (b) Schematic representation of the crystal structure of $d(CGTATACG)_2$, indicating the residues involved in junction formation. [Reproduced from Ref. 6]

the limited structural understanding of DNA junction formation and its interactions with small molecules has hindered the development of these targeted therapies.

The study by Ming-Hon Hou (National Chung Hsing University) and his team sheds light on the structural basis of DNA junction formation and provides valuable insights into targeting DNA junctions with bidirectional bis-intercalators for anticancer drug development.[6] The elucidation of the complex crystal structure required the access to a high-resolution X-ray facility housed at the NSRRC beamline **TLS 15A1**. Hou's team solved the crystal structure of d(CGTATACG)$_2$ DNA, which exhibited a unique duplex–duplex junction (**Fig. 1**). In the central region, the structure showed B-DNA-like right-handed features. Interestingly, the terminal CG base pairs contributed to forming a helix–helix junction with two tetraplex base pairs at the junction interface. Detailed analysis revealed that this structure closely resembles the duplex–duplex contacts in catenated DNA and that the tetraplex interface at the junction site serves as a "hotspot" capable of accommodating external ligands between the two neighboring duplexes.

Next, the team explored the possibility of targeting this junction structure with small-molecule compounds. Yih-Chern Horng (National Changhua University of Education) synthesized two alkyl-linked diaminoacridine compounds, DA4 and DA5 (**Fig. 2(a)**). Both DA4 and DA5 contain acridine chromophores connected by semi-flexible linkers that are four- and five-carbon long, respectively. These two acridine derivatives possess inter-duplex DNA intercalating properties. To investigate the binding mechanism of DA4 and DA5 with DNA, they determined the crystal structures of DA4 and DA5 with the d(CGTATACG)$_2$ sequence in the C222$_1$ and P2$_1$ space groups, respectively, at a resolution of 1.58 Å. As expected, in

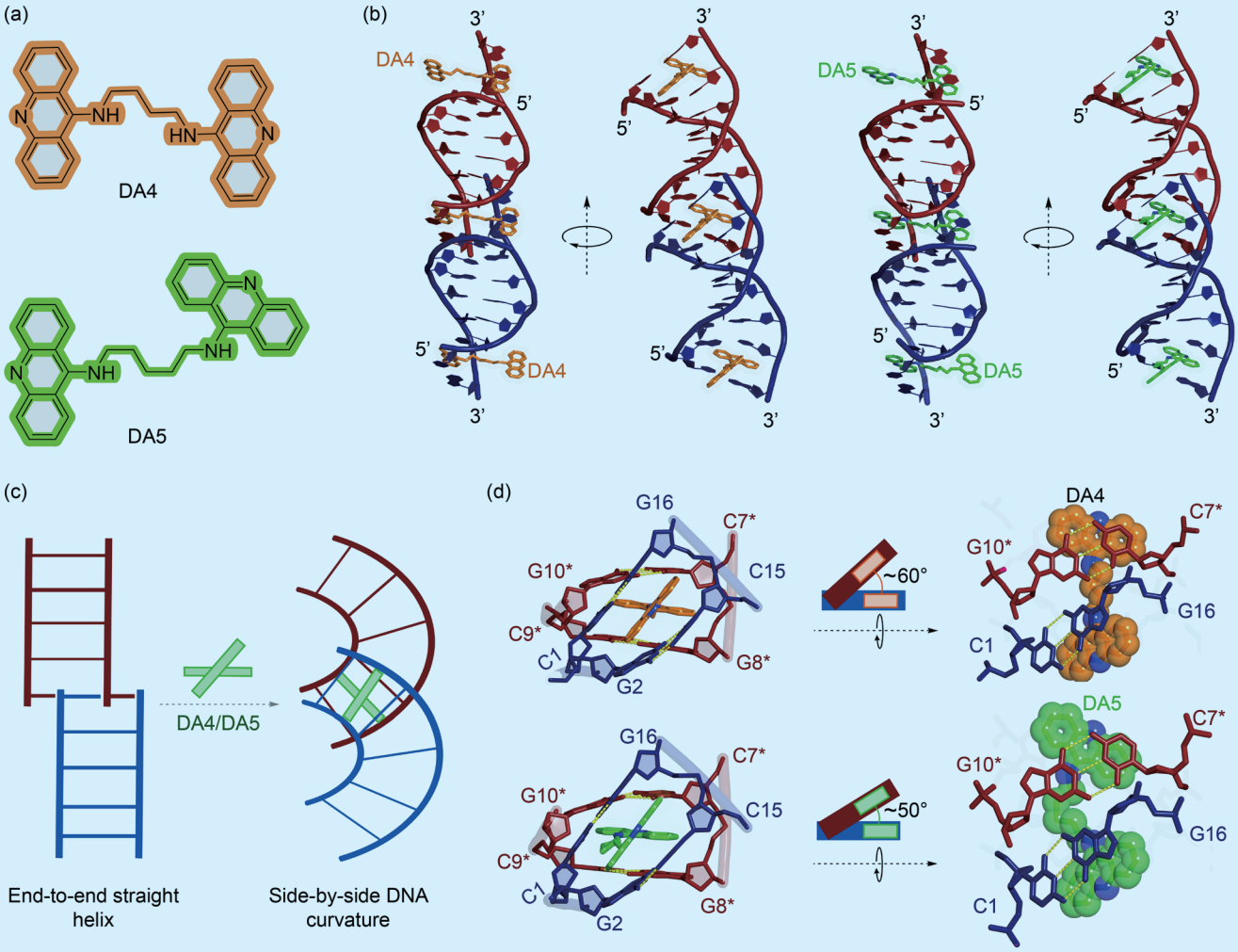

Fig. 2: (a) Chemical structures of the alkyl-linked diaminoacridine bis-intercalators DA4 (orange) and DA5 (green). (b) Crystal structure assembly of DA4–DNA and DA5–DNA complexes showing inter-duplex cross-linking of the DNA duplexes. (c) Schematic diagram showing topological changes in DNA upon intercalation of DA4 or DA5 at the terminal junction site. (d) Magnified view of the intercalation site shows the angled intercalation of DA4 and DA5 at the junction interface in steps C1pG2/C15pG16 in one duplex and C7*pG8*/C9*pG10* in the other adjacent duplex, viewed from the front and top. In DA4, the linker connecting the two acridine moieties is straight, whereas the linker in DA5 has a bent conformation. A single-atom difference in the linker of DA4 and DA5 led to distinct propeller geometries with approximately 60° and 50° between two ligands, respectively. [Reproduced from Ref. 6]

both crystal structures, the intercalation of DA4 or DA5 mediated DNA–DNA contacts and cross-linked adjacent duplexes (**Fig. 2(b)**). Compared to the unliganded native DNA junction structure, DA4 and DA5 induced significant changes in DNA topology, transforming it from an end-to-end straight helix to a side-by-side curved geometry (**Fig. 2(c)**). The cross-linking of DNA duplexes also caused a transition from the B-form to an A-form-like conformation, accompanied by bending and overwinding of the backbone. The different linker lengths and flexibilities of DA4 and DA5 resulted in distinct local structural and stabilizing effects on DNA. In the DA4–DNA complex, the four-carbon linker of DA4 adopted a straight geometry, while the DA5 linker bent toward one of the DNA backbones (**Fig. 2(d)**). This difference in linker flexibility caused the chromophores of DA4 and DA5 to stagger at different angles. In the DA4–DNA complex, the chromophores formed an angle of approximately 60° with the horizontal plane of the acridine ring, while in the DA5–DNA complex, the angle between the two acridine chromophores was approximately 50°. This flexibility of the DA5 linker allowed its chromophores to align more optimally, enabling continuous stacking interactions with DNA base pairs. Consequently, DA5 exhibited more stacking interactions with DNA than DA4. The bent linker and less tilted chromophore of DA5 brought its amino groups closer to the cytosine bases, facilitating a direct water-mediated interaction that likely resulted in a more stable complex with stronger binding. By contrast, the greater distance between the amino group of the DA4 linker and the keto group of cytosine in the DA4–DNA complex led to indirect and weaker water-mediated interactions. These findings suggest that DA5 induces stronger structural changes in DNA than DA4, potentially leading to stronger stabilizing effects. These results were further corroborated through biophysical experiments.

When tested in *in vitro* and *in vivo* models, the two acridine derivatives inhibited topoisomerase II activity, induced G2/M phase accumulation in the cell cycle, triggered apoptosis, and reduced cancer tumor growth, highlighting the anticancer potential of this mode of DNA binding. Notably, the results showed that DA5 exhibited more pronounced anticancer effects than DA4, likely due to its enhanced stability and stronger DNA-binding interactions at the junction. Through investigation of the structural basis for these results, Hou and his team demonstrated how small molecules can precisely target and stabilize DNA junction sites, inhibit topoisomerase activity, and impair cancer cell proliferation. These findings could guide the development of more effective derivatives in future, paving the way for targeting DNA–DNA duplex contacts through bis-intercalation. (Reported by Roshan Satange, National Chung Hsing University)

This report features the work of Ming-Hon Hou and his collaborators published in Nucleic Acids Res. ***52****, 9303 (2024).*

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- X-ray Crystallography
- Biological Macromolecules, Cancer, DNA Junctions, Life Science

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