**TLS 13B1 SW60 – Protein Crystallography**
**TLS 15A1 Biopharmaceuticals Protein Crystallography**

- Protein Crystallography
- Biological Macromolecule, Protein-DNA Structure, Life Science

## References

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# Parity-Dependent Slippage of DNA Hairpins for a Disease-Associated Repeat Expansion

*The structure of the repeat hairpin provides a clue to understand the initial expansion of repetitive DNA sequences associated with neurological diseases.*

Repetitive DNA sequences play a vital role in the maintenance of normal function and pathology. The expansion of DNA repeats, even in non-coding regions of the genome, might disrupt cellular replication, repair and recombination and ultimately lead to altered gene expression. DNA repeat expansions of many types are associated with neurological diseases that wreak devastating consequences.[1,2] To complicate matters further, pathological DNA expansions might occur spontaneously, so there is a great interest in understanding their mechanism.

It is generally acknowledged that hairpin loops (**Fig. 1(a)**) are critical for the expansion of repetitive DNA sequences, but the relation between the hairpin structure and the initiation of expansion remains unclear. A collaborative team led by Ming-Hon Hou (National Chung Hsing University) and I-Ren Lee (National Taiwan Normal University) combined X-ray crystallography with various biophysical methods to provide clues to this initiation.[3] They studied the behavior of a pentanucleotide TGGAA repeat hairpin, which is associated with a spinocerebellar ataxia type 31; using single-molecule fluorescence resonance-energy transfer (smFRET), they found that the hairpin was able to interconvert dynamically (slip) be-

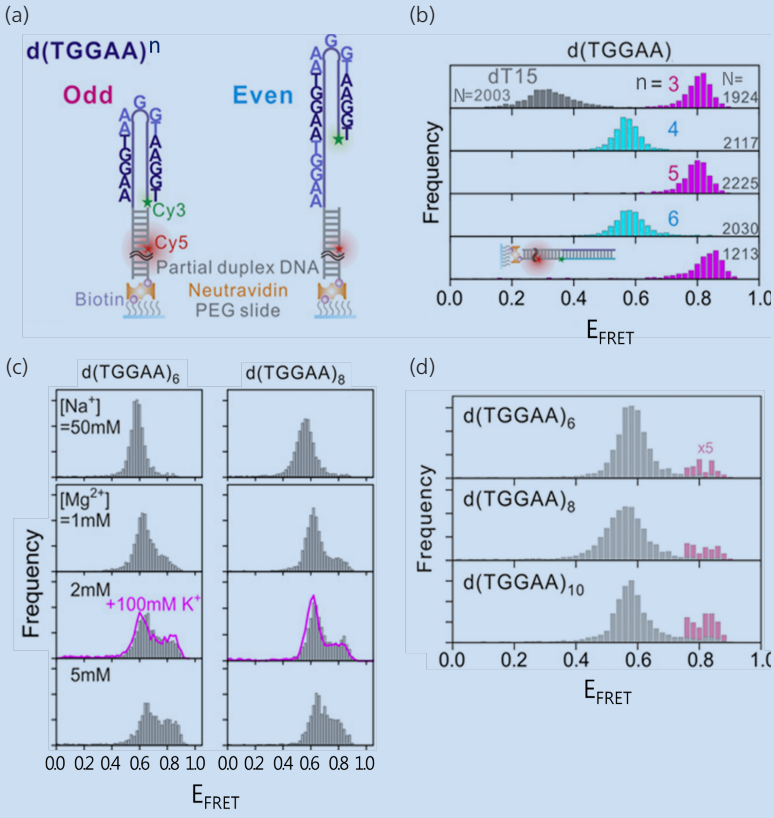


**Fig. 1**: Structural characterization of $d(TGGAA)_n$ using single-molecule FRET. (a) Illustrations of the single-molecule assay used in this experiment. (b) $E_{FRET}$ histograms of $d(TGGAA)_{3-6}$ (colored) and the assay used as a caliper of the end-to-end alignment (cartoon at bottom). (c) $E_{FRET}$ histogram of $d(TGGAA)_{6,8}$ under various salt conditions. The fractions of $E_{FRET}$ > 0.8 increase with increasing concentrations of $Mg^{2+}$. (d) $E_{FRET}$ histograms of $d(TGGAA)_n$, with n = 6, 8 and 10. [Reproduced from Ref. 1]

tween an end-to-end and an overhang configuration. Odd-numbered repeats favoured the end-to-end configuration, whereas even-numbered repeats favoured the overhang configuration (**Fig. 1(b)**). The overhang configuration was also more prone to melt, which might provide a protective effect against repeat expansion. Longer repeats allowed formation of end-to-end configurations even when the parity was unfavorable; the process could be modulated by the presence of divalent ions (**Figs. 1(c) and 1(d)**), but the physical rationale behind these observations remained elusive.

Elucidation of the physical basis would have been impractical without the structural work conducted at beamlines **TLS 15A1** and **TLS 13B1**. Hou's group solved the crystal structure of d(GTGGAATGGAAC) with the MAD method using a brominated oligonucleotide G [br5U] GGAATGGAAC.[3] The oligonucleotide formed a self-complementary antiparallel duplex that serves as a perfect representation of the stem-loop region within the TGGAA repeat hairpin. The duplex contained a tandem repeated motif in which two unpaired central guanine bases from each strand of the duplex, flanked by two sheared G·A mismatches, are intercalated and stacked on top of each other (**Fig. 2(a)**). The vertical stagger and stacking of these two unpaired guanines between the sheared G·A pairs in the two $[GGA]_2$ motifs causes the two pentanucleotide segments of the decamer duplex to kink toward the minor groove at the central A5pT6 step (**Fig. 2(b)**). This sharp kink might act as a hot spot to destabilize the duplex and to enable formation of alternative DNA structures at room temperature. Hou and his co-workers also observed that two Co(II) ions are bis-coordinated to O6 of two consecutive unpaired guanines with an incomplete hydration shell, which further stabilizes the stacking between the two unpaired guanines (**Fig. 2(c)**). The authors stipulated that the stabilization energy from the stem region counteracted the destabilization effect of the loop region in longer even-numbered TGGAA repeats, thus allowing the transition to an end-to-end configuration even when the number parity was not favourable.

As the TGGAA motif includes many structural features observed in other tri-, tetra-, and pentanucleotide repeats, the information obtained from this work might be applicable to other DNA repeats, particularly those associated with neurological disorders. In addition, the dependence of the slippage phenomenon on the divalent ion might provide a way to manipulate the process and to extend its applicability to the development and nanotechnology of DNA-based sensors. (Reported by Chun-Jung Chen)

*This report features the work of Ming-Hon Hou, I-Ren Lee, and their collaborators published in PNAS* ***114****, 9535 (2017).*

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## References


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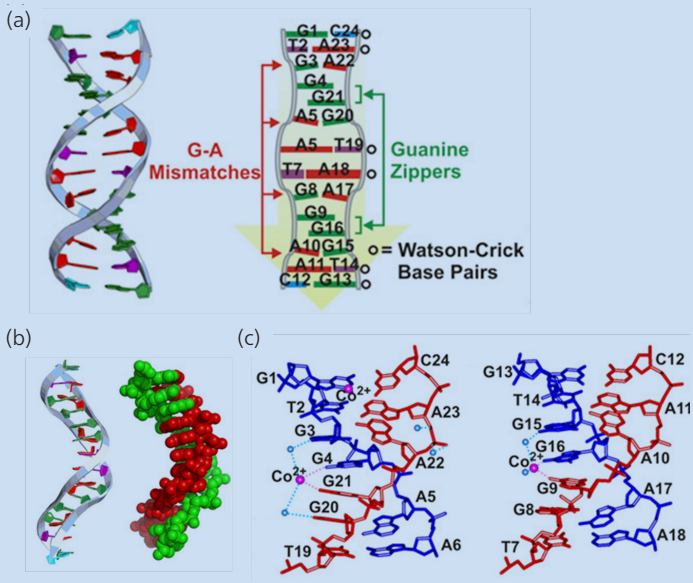


**Fig. 2**: (a) Structure of the dG(TGGAA)2C duplex. (b) Side view of the dG(TGGAA)2C crystal structure in ribbon form. Close-up view of the molecular structure of dG(TGGAA)2C at the (c) G1 to A6 (left) and G13 to A18 (right) terminal base-pair steps. [Reproduced from Ref. 1]