NMR REVEALS DYNAMIC G-QUADRUPLEX DNA MODULATION BY METHYLATION AND MOLECULAR INTERACTIONS,

Janez Plavec [a,b,c]

[a] Slovenian NMR center, National Institute of Chemistry, Ljubljana, Slovenia
[b] University of Ljubljana, Faculty of Chemistry and Chem. Techn., Ljubljana, Slovenia
[c] EN-FIST Centre of Excellence, Ljubljana, Slovenia
janez.plavec@ki.si

Noncanonical DNA structures include G-quadruplexes (G4) - four-helical structures that may form when the sequence contains more than four tracts of guanine bases. Cytosine methylation, a key epigenetic modification that regulates gene expression, has drawn interest for its potential impact on G4 structures and their stability. Using NMR spectroscopy—a powerful tool for probing structural dynamics—we examined how methylation affects the well-characterized bcl2Mid G4, which is involved in regulating BCL-2 gene expression. By combining solution-state NMR with other biophysical methods, we found that cytosine methylation induces local rearrangements within the bcl2Mid G4, leading to changes in its thermodynamic stability. Unexpectedly, methylation not only modifies stability but also alters the folding pathway by which the G4 adopts its predominant form. Specifically, methylation shifts the equilibrium between the major G4 conformation and a previously unidentified minor form. In some cases, the methylated sequence even favors the minor form more strongly than the unmethylated counterpart. Overall, our results demonstrate that cytosine methylation does more than fine-tune G4 stability—it can act as a molecular switch that redirects G4 folding pathways, with potentially significant consequences for gene regulation.[1]

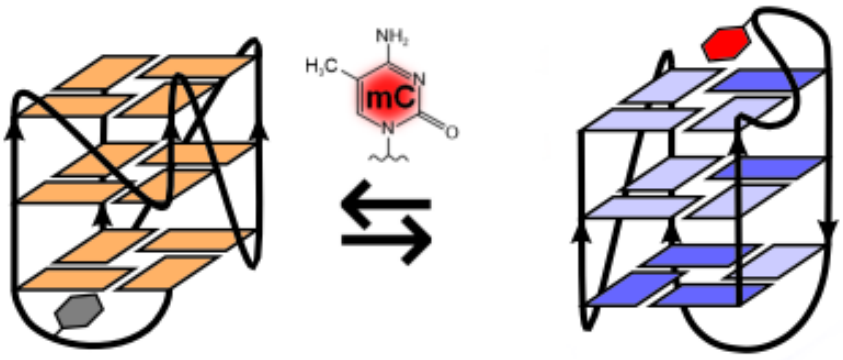

Figure 1. G4 structures adopted by the G-rich sequence originating from the BCL-2 promoter region whose equilibrium between the major (3+1) hybrid and the minor parallel topology is controlled by 5-methylcytosine substitution.

REFERENCES

[1] N. Medved, M. Cevec, U. Javornik, J. Lah, S. Hadži, J. Plavec, *Angew. Chem. Int. Ed.* **2025**, 64, e202507544.