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Bio-Layer Interferometry (BLI): a useful tool to investigate the interactions of i-motif DNA with small molecules and proteins

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i-Motifs of DNA (hereafter, *i*-DNA), known *in vitro* for nearly three decades, are unusual, four-stranded structures, in which cytosines are intercalated *via* a stack of hemi-protonated C–C base pairs (CH⁺:C) (Fig. 1A, B). Some of these structures have been well characterized *in vitro* and, because *i*-DNA may mirror other four-stranded G-rich structures (G-quadruplexes) present in gene promoters or at telomeres, their biological relevance is being investigated.

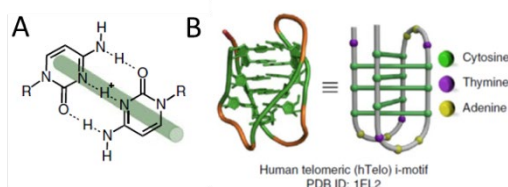


Figure 1. A/ Hemi-protonated C-C base pair. B/ Schematic structure of the telomeric *i*-DNA.

Relatively few molecules were reported to interact with *i*-DNA. The main issues in this regard are the strong pH-dependency, flexibility, polymorphism and complex folding behavior of *i*-DNA, which introduce potential bias into screening methods. In particular, low-pH conditions used to induce the formation of *i*-DNA lead to the protonation of many ligands, which can strongly increase their non-specific nucleic acid binding. This latter point is particularly critical because the use of small molecules to study the biological functions of such structures is essential.

In this context, we have developed a method using the Bio-Layer Interferometry (BLI) to screen and to study the interactions between the *i*-motif and already reported ligands (TMPyP4, mitoxantrone, IMC-48, berberine, *etc*) at physiologically relevant pH. We demonstrated that none of the reported ligands were shown to discriminate between folded and unfolded *i*-motif structures.[1,2]

References

- [1] Bonnet, H.; Morel, M.; Devaux, A.; Boissieras, J.; Granzham, A.; Elias, B.; Laverigne, T.; Dejeu, J.; Defrancq, E. Assessment of presumed small-molecule ligands of telomeric *i*-DNA by biolayer interferometry (BLI). *Chem. Commun.* **2022**, *58*, 5116-5119.
- [2] Berthiol, F.; Boissieras, J.; Bonnet, H.; Pierrot, M.; Philouze, C.; Poisson, J.-F.; Granzham, A.; Dejeu, J.; Defrancq, E. Novel Synthesis of IMC-48 and Affinity Evaluation with Different *i*-Motif DNA Sequences. *Molecules* **2023**, *28*, 682.