BLI used to characterize the interaction between antibodies and non canonical DNA conformation

Jérôme Dejeu
Université Marie et Louis Pasteur, SUPMICROTECH, CNRS, institut FEMTO-ST, F-25000 Besançon, France
Jerome.dejeu@femto-st.fr

Genomic DNA has the capacity to form alternative structures to the canonical double helix. Among them, we can cite: i) G-quadruplexes (G4s) are tetrahelix structures that arise from G-rich genomic regions and result from the self-assembly of guanine residues into quartets, which are further stabilized by π-stacking interactions and coordination with metal cations such as K^+ or Na^+ and ii) *i*-Motifs (hereafter, *i*-DNA) are four-stranded structures, in which cytosines are intercalated *via* a stack of hemi-protonated C–C base pairs (CH^+:C). They are known to play crucial roles in various cellular processes, including transcription regulation, DNA replication, and telomere maintenance. Dysregulation of these structures has been linked to several diseases, such as cancer and neurodegenerative disorders.

Numerous *in vitro* studies have shown that G4s are highly susceptible to adopt multiple topologies, which exist in dynamic equilibrium. To investigate the structural and functional properties of G4 DNA in cells, few G4 antibodies have been identified. Most of them, including commercially available BG4, recognize the G4 structure versus duplex DNA but are not specific for a particular topology, in particular to differentiate between parallel and antiparallel G4 conformations[1]. We used the Elisa and the Bio-Layer Interferometry for the studies of the affinity of two selected antibodies and we have demonstrated that these two new antibodies are highly selective for G4 telomeric DNA versus duplex and single-stranded DNA.

For the i-DNA, only one antibody is available on the market (*i-Mab*), that was instrumental for several studies that suggested the existence of iMs in live cells[2]. We are repeated and completed the previous BLI experiments by adding several controls and different conditions. With the new study, the i-Mab was no specific to the i-DNA and the binding of iMab to DNA oligonucleotides is governed by the presence of runs of at least two consecutive cytosines and is generally increased in acidic conditions, irrespectively of the capacity of the sequence to adopt, or not, an iM structure[3].

References

[1] Biffi G, Tannahill D, McCafferty J, Balasubramanian S. Nature Chem. **5**, 182 (2013).
[2] Z eraati,M., Langley,D.B., Schofield,P., Moye,A.L., Rouet,R., Hughes,W.E., Bryan,T.M., Dinger,M.E. and Christ,D. *Nat. Chem.*, **10**, 631 (2018).
[3] Boissieras J, Bonnet H, Fidelia Susanto M, Gomez D, Granzhan A, Defrancq E, Dejeu J. Nucleic Acids Res. **52**, 8052 (2024).

Acknowledgements

We thank the Agence Nationale de la Recherche (grants ANR-16-CE11-0006-01 and ANR-21-CE44-0005-02), the University Grenoble Alpes Graduate School (ANR-17-EURE-0003), the EIPHI Graduate School [ANR-17-EURE-0002] and the CNRS through the MITI interdisciplinary program (PRIME _ 80, project LiDNA) for financial support. NanoBio-ICMG (UAR 2607) is acknowledged for their support for BLI studies. We acknowledge TRI-IPBS imaging facility, member of the national infrastructure France-BioImaging supported by the French National Research Agency (ANR-24-INBS-0005 FBI BIOGEN)".