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G-quadruplex denaturation and stabilization: interaction with helicases and screening of G4 ligands

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G-quadruplexes are highly polymorphic non-canonical nucleic acid structures adopted by both DNA and RNA guanine-rich sequences in vitro. They have been detected at the cellular level using structure specific antibodies and small molecule ligands. Computational studies demonstrated that G4-prone sequences are located in key genomic regions such as telomeres and oncogene promoters. Numerous studies showed that G4 sequences can interact with proteins involved in cellular processes, including replication, transcription or reparation. Those interactions include binding, G4 folding promotion or in contrary unwinding. Indeed, WRN, BLM, FANC J or Pif1 are helicases associated with human-diseases. They can unwind G4 forming sequences; mutation of these helicases lead to genomic instability of G4-prone motifs when mutated.

Here, we present a medium-throughput technique to monitor G4-helicase interactions in real time. We were able to determine both favourable and deleterious conditions for G4 unwinding by a given helicase. We show that these conditions differ from one helicase to another as exemplified with the optimal salt conditions required for both ScPif1 and RHAU activities. We also reveal that the G4 ligands that stabilize G4 structures do not necessarily induce an inhibition of their unwinding by ScPif1 helicase. Finally, we also prove that our assay is adapted to clear up RPA directionality, making it an attractive technique to screen for new proteins able to unwind G4 structures.