ID de Contribution: **47** Type: **Présentation orale**

Folding and persistence times of intramolecular G-quadruplexes transiently embeded in a DNA duplex

G-quadruplex (G4) DNA structures have emerged as important regulatory elements during DNA metabolic transactions. Most *in vitro* studies have focused so far on the G4 kinetics within single-stranded DNA. However, G4 structures potentially form in genome regions where their stability is challenged by a complementary strand. Since the energy of hybridization of Watson-Crick structures dominates the energy of G4 folding, this competition should play a critical role on the persistence of G4 structures *in vivo*. Here, we addressed the kinetics of G4 folding and unfolding in presence of a complementary strand. We designed a single molecule assay allowing measuring G4 folding and unfolding while the structure is periodically challenged by the complementary strand. By repeating cycles of opening and closing of the DNA duplex, we quantified the folding rate and the persistence time of biologically relevant structures from three different genomic origin, namely human oncogene promoters, human telomeres and an avian replication origin. We show that the dynamics of G4 formation varies largely in ways not fully predictable by current knowledge. In addition, we show that folding and persistence time increase upon treatment with a G4 binding ligand or an anti-G4 antibody. Our assay opens new perspectives for the measurement of G4 dynamics in vitro in biologically inspired substrates.

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