Multi-scale conformational dynamics of G-quadruplex DNA studied by time-resolved circular dichroism

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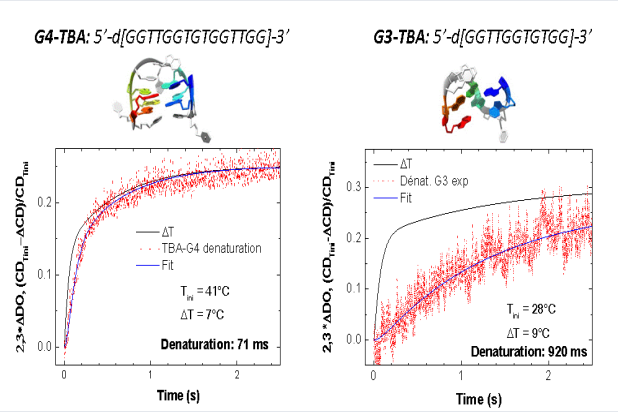
Besides their 3D structure, the structural dynamics of biomolecules play a key role in their functions. During their folding mechanism, they are able to explore a rich set of conformations that is difficult to capture experimentally. In this regard, circular dichroism (CD) provides a valuable tool for investigating structural changes over large time scales. Time-resolved experiments remain however technically challenging, due to extremely low signals. In recent years, we have developed two complementary monochromatic time-resolved CD (Tr-CD) set-ups allowing the monitoring of conformational changes of biomolecules over a time scale ranging from a few hundred femtoseconds up to the millisecond. In the frame of QUADfold, we developed a novel extension of our Tr-CD set-ups capable to measure the thermal denaturation and the consecutive renaturation of peculiar DNA structures, the G-quadruplexes (G4), over a time window extended up to seconds.

G4 are non-canonical DNA structures resulting from the hydrophobic stacking of a number of guanine quartets in the presence of metallic cations such as K^+ or Na^+. There are compelling experimental evidences of their participation in important cellular regulation functions correlated to their folding mechanism. We measured the folding kinetics of several G4-forming sequences made of ~20 bases, with a combination of steady state CD and Tr-CD CD spectroscopy. Their denaturation and renaturation kinetics were found to occur within a few tens of milliseconds and suggest the existence of multiple folding routes along very flat and rugged energy landscapes that strongly depend on the concentration of metallic cations. The comparative study of the thrombin aptamer G4 forming sequence with the associated G-triplex-forming sequence highlighted the slower formation of the triplex topology with respect to that of the G4. This important result questions the frequently made assumption that a G-triplex intermediate is formed in the course of antiparallel G4 folding. Additional measurements of thermal denaturation of G4 and their interaction with small organic ligands in the femtosecond-nanosecond time scale are currently under way at LOB and LIDYL.



Thermal denaturation kinetics of two thrombin aptamer sequences in 10 mM phosphate buffer at pH7, with 150mM K+, measured at 293 nm after fast T-jump (ΔT).

Marco Schmid, Thèse de doctorat de l' Université Paris-Saclay (2017), Conformational dynamics of G-quadruplex DNA probed by time-resolved circular dichroism. 2017SACLX107

P. Changenet-Barret, M. Schmid, F. Hache, S. Guy, L. Martinez, R. Improta, D. Markovitsi, Unveiling excited-state chirality of binaphthols by sub-picosecond circular dischroism and quantum-chemical calculations, to be submitted.

M. Schmid, F. Wien, F. Hache, P. Changenet-Barret, Denaturation and renaturation dynamics of antiparallel thrombin aptamer G-quadruplex and G-triplex probed by time-resolved T-jump and circular dichroism spectroscopy, in preparation.

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