Dynamique multi-échelles de repliement de l'ADN G-quadruplexe sondées par dichroïsme circulaire résolu en temps

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Résumé :

G-quadruplexes (G4) are non-canonical DNA structures involved in important biological functions related to their folding mechanisms. Recent molecular dynamics simulations suggest that these processes may involve complex pathways over time scales spanning several orders of magnitude and several intermediates.

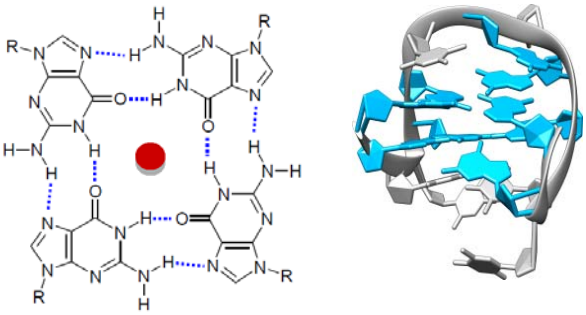
The objectives of the present PhD project aim at exploring experimentally the entire folding mechanism of G4 and identifying the parameters controlling the reaction. We will use a combination of circular dichroism (CD) spectroscopies to assess the structural changes of DNA over a timescale spanning picoseconds to seconds. CD spectroscopy is a very sensitive probe of G4 topologies, providing precise information about the chiral arrangement of their guanines constituents in the DNA scaffold. DNA denaturation will be initiated by fast laser-induced temperature jump (T-jump). In addition, collaboration with SOLEIL Synchrotron will allow the acquisition of the entire CD spectra of G4 (<180 nm) with a time-resolution of a couple of microseconds, providing the opportunity to identify putative folding intermediates.

The samples will be short single-stranded G4 model structures composed of ca. 20 bases, representative of various topologies. The effects of the environment (nature of cations, ionic strength and crowding effects) will be studied to determine how they affect the thermodynamic stability of G4.

Keywords : DNA, G-quadruplex, Circular dichroism, Transient absorption, Femtosecond

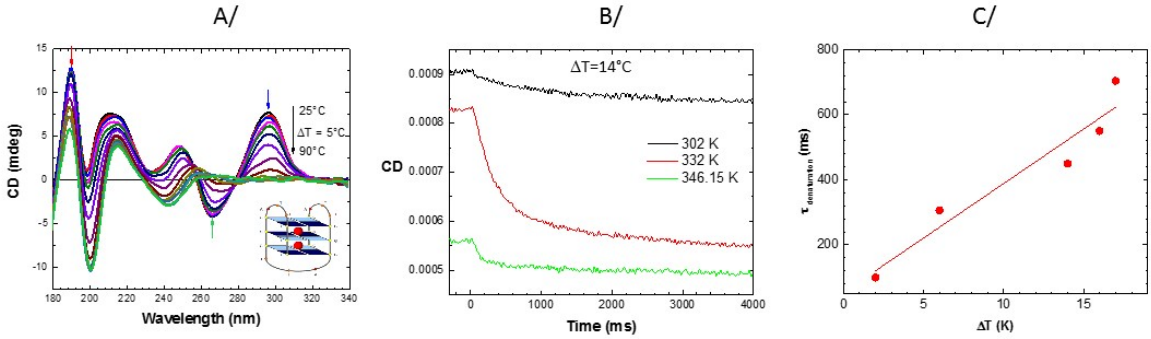
Sujet de recherche :

G-quadruplexes (G4) are non-canonical DNA structures involved in important biological functions related to their folding mechanisms. Recent molecular dynamics simulations suggest that these processes may involve complex pathways over time scales spanning several orders of magnitude and several intermediates. However, the only experimental studies that addressed this question have a limited temporal resolution (microseconds or larger) and a limited structural resolution, leading to contradictory pictures of the folding processes.



Schematic representations of a G-quartet with metal cation in red and of antiparallel thrombin aptamer G4 structure.

The objectives of the present PhD project aim at exploring experimentally the entire folding mechanism of G4 and identifying the parameters controlling the reaction. Project incentive will focus on the transversal use of a combination of circular dichroism (CD) spectroscopies to assess the structural changes of DNA, over a timescale spanning more than ten orders of magnitude. CD spectroscopy is a very sensitive probe of G4 topologies, providing precise information about the chiral arrangement of their guanine constituents. Notably, the development of unique time-resolved chiroptical tools at LOB makes the direct observation of the conformational dynamics involving the secondary structure of DNA possible, over a time scale spanning picoseconds to seconds. In addition, collaboration with SOLEIL synchrotron will allow the acquisition of the entire CD spectra (SRCD) of G4 (<180 nm) with a time-resolution of a couple of microseconds, providing the opportunity to identify the folding intermediates. DNA denaturation will be initiated by fast laser-induced temperature jump (T-jump) achieved by the direct excitation of the water solvent at 1.5 µm. These experiments that rely on the return to equilibrium of a system after a rapid increase of the temperature will provide a measure of both the folding and unfolding rates of DNA, with an ultimate resolution of a few picoseconds corresponding to the thermal limit of water heating.



A/ SRCD denaturation spectra of Tel21, from 25 to 95°C. Inset: schematic representation of the folding topology of Tel21, in aqueous solution with Na^+ (red circles); guanines in the syn and anti conformation are denoted, respectively, by light and dark blue squares. (B) CD changes of Tel21, probed at 293 nm, after a 14°C T-jump, for three initial temperatures. (C) Variations of the observed denaturation times of Tel21, for increasing T-jumps, for initial temperatures close the melting temperature at 332 K. (Unpublished results)

The PhD work will focus on short single-stranded G4 model structures composed of ca. 20 bases. Due to their large structural diversity, biologically relevant and structurally well-

characterized sequences will be selected. In addition to the primary sequence, the effect of the glycosidic bond angles of their guanine constituents as well as of environmental factors (ionic strength, nature of cations, crowding effects) known to affect the thermodynamic stability of G4 will be examined.

This project is highly multidisciplinary. The PhD student will participate to the selection, the preparation and the characterization of the G4 samples as well as to the state-to-the-art time-resolved CD experiments. By providing a global picture of the structural dynamics of various G4 model structures, this project aims to establish a structural mapping of the conformational changes of DNA that will contribute to a better understanding of the biological functions of G4.

References

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