Single Molecule Force Spectroscopy (FS) studies of G-quadruplexe DNA conformations

Keywords: Force spectroscopy, AFM, G-quadruplexes

Contact : Jerome.dejeu@univ-grenoble-alpes.fr, eric.defrancq@univ-grenoble-alpes.fr

G-quadruplex DNA consists in a four-stranded structure of stacked guanine tetrads formed by the coplanar arrangement of four guanines, and held together by Hoogsteen bonds. This particular DNA topology (*versus* classical double-stranded DNA structures, *i.e.* duplex DNA) has found an increasing interest due to their implication in several biological processes (*e.g.* oncogenes control and telomere organization). G-quadruplexes show an **important polymorphism** (*i.e.* presence of different topologies in which the strands are in parallel or antiparallel conformations, with the co-existence of different types of loops) that is considered as a crucial issue for designing new ligands and studying their interactions with a given topology. In this context, we have developed an original concept that consists in constraining the accessible topologies of a G-quadruplex-forming sequence by using a cyclic peptide scaffold as a topological template for directing the intramolecular assembly of the anchored oligonucleotides[1]. Different constrained G-quadruplexes including parallel G4-DNA[2] and G4-RNA, DNA-RNA hybrid G4[3], antiparallel loop containing G4-DNA (Telomeric and HIV sequences[4]) have been prepared by using appropriate ligation methods for anchoring the oligonucleotides strands onto the template. Those systems are currently used for investigating the interaction of ligands (small molecules as well as proteins) with a given topology by using surface plasmon resonance (SPR) and Bio-Layer Interferometry (BLI) to obtain the kinetic and thermodynamic parameters of these interactions.

Single molecule (SM) force spectroscopy (FS), based on **atomic force microscopy (AFM)** has been demonstrated as a powerful tool for measuring the interactions forces at molecular level in biological systems. The major advantage is the direct measurement using unlabeled individual single molecules, one at time, avoiding ensemble averaging. Furthermore, the recent development of theoretical analysis for single molecule approaches has enabled reliable estimates of the kinetic and thermodynamic parameters.

To the best of our knowledge, the **AFM SM-FS has scarcely been used to study G-quadruplex (less than 5 papers).** In this context, our constrained G-quadruplex systems represent interesting tools for investigating the intramolecular forces (π-staching as well as hydrogen bonds) involved in the formation of the G-quadruplex. This master research project aims at studying bi-molecular G-quadruplex formed between the AFM probe and a gold surface (Fig 1) and investigates the effect of strand orientation (parallel, antiparallel) by using the appropriate constrained system. These studies will provide unique insights such as structure/stability relationship for distinct G4 topologies and will afford interesting data that could be used to inform on their biological function as well as for applications in nanotechnology.

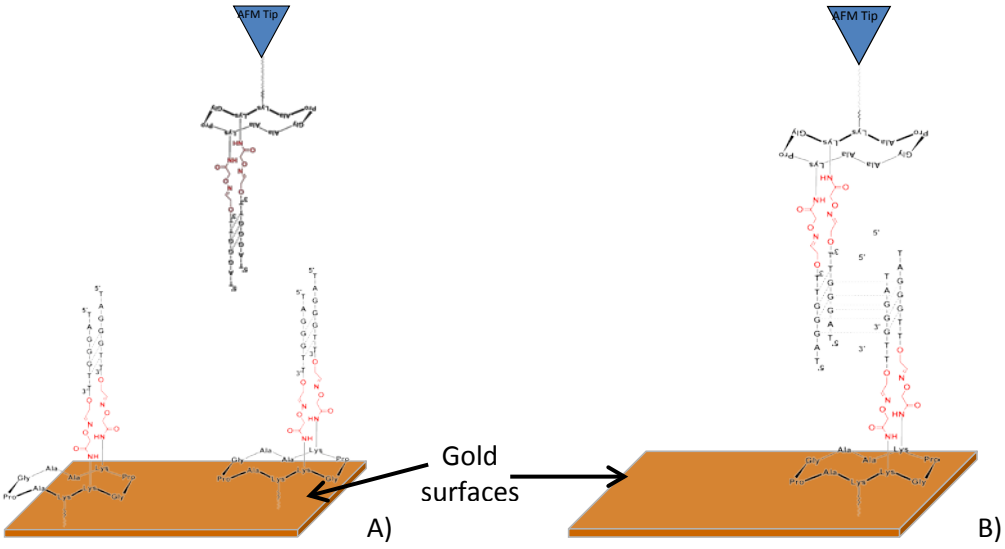

Figure 1: Schematic figure of the AFM SM-FS study on the G-quadruple formation: A) Starting, B) G4 formation

The student will have to know the AFM and if possible of the knowledge in force spectroscopy and in Matlab

[1] P. Murat, D. Cressend, N. Spinelli, A. Van der Heyden, P. Labbé, P. Dumy, E. Defrancq, *ChemBioChem*, 9 **(2008)** 2588.
[2] L. Sabater, P.-J. Fang, C.-F. Chang, A. De Rache, E. Prado, J. Dejeu, A. Garofalo, J.-H. Lin, J.-L. Mergny, E. Defrancq, G. Pratviel, *Dalton Trans.* 44 **(2015)** 3701; L. Lecarme, E. Prado, A. De Rache, M.-L. Nicaulo-Travers, G. Gellon, J. Dejeu, T. Lavergne, H. Jamet, D. Gomez, J.-L. Mergny, E. Defrancq, O. Jarjayes, F. Thomas, *ChemMedChem* 11 **(2016)** 1133 ; J. Dejeu, T. Lavergne, J. Della Nora, E. Defrancq, G. Pratviel, *Inorg. Chim. Acta* 452 **(2016)** 98.
[3] L. Bonnat, J. Dejeu, H. Bonnet, B. Génnaro, O. Jarjayes, F. Thomas, T. Lavergne, E. Defrancq, *Chem. Eur. J.* 22 **(2016)** 3139
[4] L. Bonnat, L Bar, B. Génnaro, H. Bonnet, O. Jarjayes, F. Thomas, J. Dejeu, E. Defrancq, T. Lavergne, *Chem. Eur. J.* 23 **(2017)** 5602.