Structural dynamics of protein-DNA complexes

DIRECTEUR DE THESE : STEFAN HAACKE
IPCMS, 23, RUE DU LOESS, 67034 STRASBOURG
TEL : 03 88 10 71 71 ; E-MAIL : HAACKE@IPCMS.U-STRASBG.FR

In biophysics and in molecular biology, knowing the microscopic structure of proteins, DNA and of the complexes they form is a very fundamental and important information, in order to understand how these molecules work. But the structure is not static: The molecules can adopt different conformations depending on whether they are biologically active or inactive. Hence, new physical methods are needed to evaluate the statistical distribution of the different conformations.

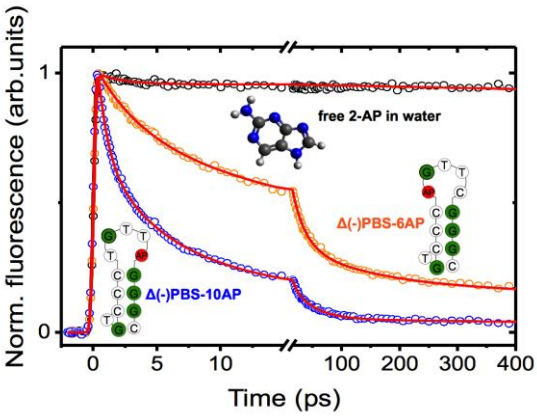

Figure 1 : Ultrafast fluorescence kinetics of the HIV primary binding sequence (PBS) labelled with the fluorophore 2-aminopurine. Compared to 2-AP in H_2O, the fluorescence is faster due to the proximity of G bases. A detailed analysis of the multi-exponential decay reveals different molecular conformations.

Since, the light absorption and fluorescence properties of biomolecules change when they undergo structural changes and form complexes, one can use laser spectroscopy for monitoring the structure of biomolecules. In our group, we are using time-resolved fluorescence and absorption for the investigation of DNA-protein complexes. We observe that the fluorescence decreases, when an amino acid like tryptophan, or a fluorescent DNA base like 2-aminopurine come in close contact, in so-called stacking interaction, with other nucleobases of DNA. The reason for this decrease is not entirely known. A transfer of a charge is held responsible, but this hypothesis needs to be tested.

The biological context of this ANR funded project is the replication of the HIV or other retroviruses and the search for a possible therapy targeting the protein NCp7. The project is therefore carried out in collaboration with the Laboratory for Biophotonics and Pharmacology at UdS (Pr. Y. Mély) and with the IGBMC Strasbourg (Dr R. Stote). Our first results on isolated DNA molecules show that measuring the ultrafast fluorescence kinetics with a high signal-to-noise ratio allows to distinguish different quenching mechanisms acting on time scales from 0.8 ps to 2-3 ns (fig. 1). The fastest quenching reveals stacking interactions, while longer ones are due to restricted local motion of the bases, leading to momentary encounters between quenching partners (diffusion-limited collisional quenching) [1].

The PhD project will build on these results, investigate the key question of how proteins change these DNA conformations, and will try to find experimental evidence for the charge transfer processes. This would allow to establish the ultrafast spectroscopy as a general method, complementary to NMR, for characterizing structurally dynamic DNA/peptide complexes. A theoretical description of the stacking interactions is also aimed for.

[1] "Ultrafast Site-specific Fluorescence Quenching 2-Aminopurine in a DNA hairpin studied by femtosecond down-conversion", ", T. Gelot, P. Touron-Toucéda, O. Crégut, J. Léonard, S. Haacke, J. Phys. Chem. A, (2012), **DOI:** 10.1021/jp212187m.