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## **Interaction du rayonnement UV avec des séquences de l'ADN riches en guanines étudiées par spectroscopie optique**

### **Spectroscopic study of the interaction between UV light and guanine-rich DNA sequences**

PhD thesis performed at Laboratoire Francis Perrin, CEA Saclay, France, supervised by Dimitra Markovitsi and Thomas Gustavsson.

Thesis defended the 14<sup>th</sup> of December 2009 before a jury consisting of Sandra Monti (CNR, Bologne, Italy), Roberto Improta (CNR, Naples, Italy), Irene Burghardt (Ecole Normale Supérieure, Paris), Rachel Meallet-Renault (Ecole Normale Supérieure, Cachan), Isabelle Lampre (Laboratoire de Chimie Physique, Orsay), Dimitra Markovitsi and Thomas Gustavsson.

F.-A. Miannay received the degree of Ph. D. at the University of Paris-Sud XI with the highest marks.

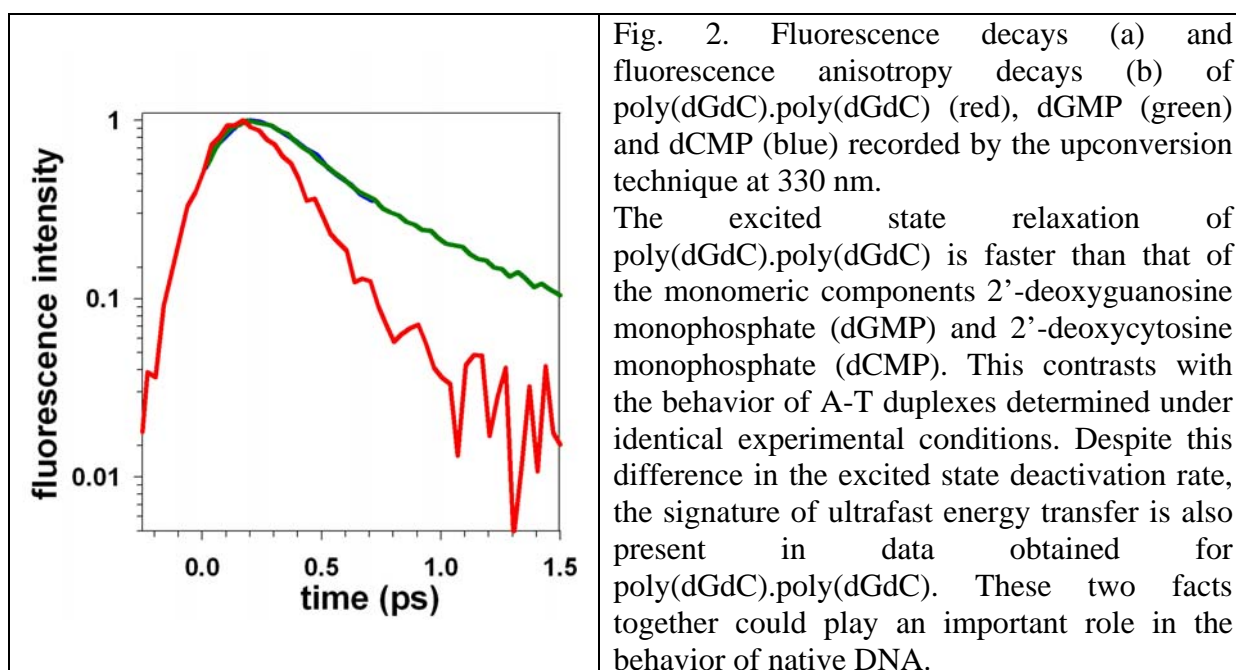
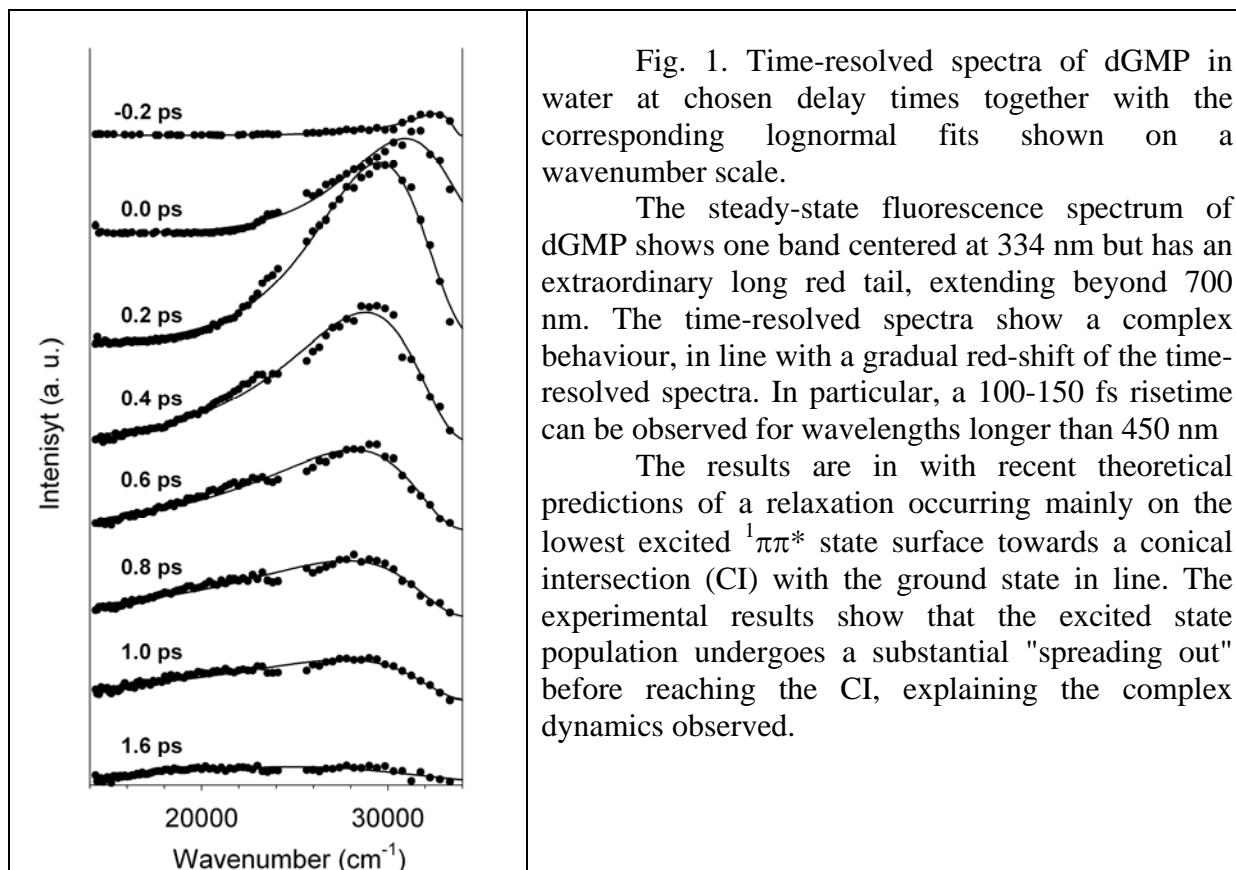
#### Abstract

It is well established that absorption of UV radiation by DNA induces photochemical reactions that ultimately may lead to carcinogenic mutations. Although the final photoproducts associated with the DNA lesions are well characterized, the fundamental processes at the origin of the photodegradation are not yet understood. The aim of this thesis was to characterize the singlet excited states of guanine-rich DNA strands. No detailed spectroscopic information was available for such systems prior to the present work.

The thesis begins with an in-depth study of the photophysical properties of the monomeric chromophore dGMP (2' deoxyguanosine 5' monophosphate) (Figure 1) [1]. Subsequently, two different types of DNA secondary structures containing GC base pairs are studied. This part starts with double helices having an alternating sequence (GC and ATGC) (Figure 2) [2]. For comparison, natural DNA double helices with randomized sequences. Finally, G-quadruplex structures formed by association of four oligonucleotides d(TGGGGT) are studied (Figure 3) [3]. All studies were performed in aqueous solution using optical spectroscopy. The excited states relaxations dynamics, as manifested by time-resolved fluorescence, were followed over a very large time scale (from 100 femtoseconds to 100 nanoseconds) by combining the two techniques "fluorescence upconversion" and "time correlated single photon counting". It is worth-noticing that the fluorescence quantum yield of DNA components is of the order of  $10^{-4}$ , making these experiments particularly delicate.

To resume, the comparison between monomeric chromophores and DNA multichromophoric systems led to the conclusion that the Franck-Condon excited states are delocalized and subject to ultrafast (<100 fs) energy transfer between the bases. It was also demonstrated that, contrary to the monomers, double and quadruple strands possess long lived emitting states, characteristic for "forbidden" transitions. Interestingly, the "normal" fluorescence, corresponding to allowed transitions, decays more rapidly for GC double strands than for G-quadruplexes whereas the opposite is observed for the "forbidden" transitions. Finally, the theoretical prediction that GC base pairs would act as energy sinks in

photoexcited DNA double strands was examined. It was shown that GC base pairs may act as "partial" energy sinks for the excited states involved in the strong fluorescence (short-lived but allowed transitions), but they cannot be considered as "absolute" energy sinks as evidenced by the existence of a non-negligible contribution of weaker fluorescence ("forbidden" but long lived transitions) emanating from high-lying excited states. The exact nature of these highly situated excited states remains to be determined.



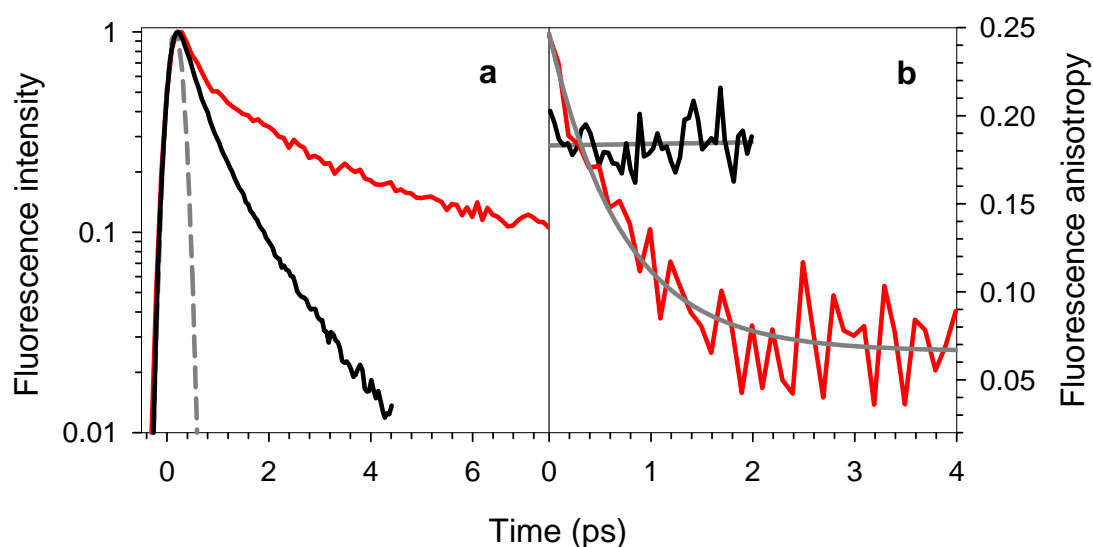


Fig. 3. Comparison of the fluorescence decay (a) and fluorescence anisotropy decay (b) of four-stranded TG4T (red) with the decays of the stoichiometric mixture of non-interacting nucleotides (black). The apparatus function is shown in dashes. Excitation wavelength: 267 nm; emission wavelength: 360 nm. The time-zero is defined at the half rise of the fluorescence decays. The grey lines correspond to fits with mono-exponential functions.

A comparison between the four-stranded structures and the corresponding stoichiometric mixture of non-interacting nucleotides shows how horizontal and vertical organization affects the properties of the excited states. Quadruplex fluorescence arises from a multitude of excited states generated via electronic coupling between guanines; the average fluorescence lifetime is longer and the fluorescence quantum yield higher compared to those of non-interacting nucleotides. The fluorescence anisotropy recorded on the sub-picosecond time-scale, where molecular motions are hindered, reveals that energy transfer takes place among the bases composing the nanostructure. These results are in line with the conclusions drawn from similar studies on model DNA duplexes.

## References

- [1] F.-A. Miannay, A. Banyasz, T. Gustavsson, D. Markovitsi, Ultrafast Excited-State Deactivation and Energy Transfer in Guanine-Cytosine DNA Double Helices, *J. Am. Chem. Soc.* 129 (2007) 14574-14575.
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- [3] F.-A. Miannay, T. Gustavsson, A. Banyasz, D. Markovitsi, Excited state dynamics of dGMP measured by steady-state and femtosecond fluorescence spectroscopy, *J. Phys. Chem. A* (accepted for publication)