Université Nice Sophia Antipolis

Institut de Chimie de Nice

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Institut de Chimie de Nice – UMR7272

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Concern: Proposal for a PhD funding starting in October 2016

Title: Synthesis and study of new fluorescent probes for labelling nucleic acids and life sciences

Presentation of the research group: The "Sondes Fluorescentes" Group at the "Institut de Chimie de Nice - UMR 7272 " is interested in the design and the chemical synthesis of fluorescent innovative dyes for DNA labeling. The laboratory of our Team "Molécules Bioactives", recently renovated, presents optimal and efficient conditions to perform research at the cutting edge of technology (self-service facilities: LCMS, NMR 200-500 MHz, Combi-Flash, HPLC, Spectrophotometer UV, Spectrofluorometer, Microwave apparatus, Flow chemistry...).

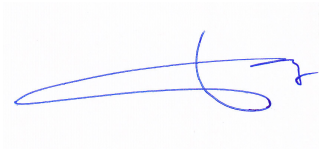
Many fundamental cellular events such as replication, translation, transcription, gene silencing, DNA repair and methylation involve complex machineries of nucleic acids and proteins. Due to the capability of fluorescence-based techniques to decrease the measurements down to a single molecule and to the exquisite sensitivity of many fluorophores to their environment, fluorescence spectroscopy is a technique of choice to unravel the molecular mechanisms and dynamics of these machineries. However, this method is limited by the availability of optimized fluorophores that sensitively, site-specifically monitor the conformational changes of nucleic acids during interaction with their protein partners.

In this context, we have developed different approaches to label DNA. Among them, environmental sensitive fluorescent dyes of the 3-hydroxychrome (3HC) family were introduced to label DNA and study with state-of-the-art fluorescence techniques, the dynamics and mechanism of DNA/protein interactions. Due to an excited state intramolecular proton transfer (ESIPT), these fluorophores exhibit two excited state forms, each emitting light with different energy (green and yellow). The dual emission of 3HCs is highly sensitive to the environment, which is reflected by a variation of the intensity ratio of the 2 bands. Therefore, this change of colour becomes easily detectable and quantifiable. We have exploited these peculiar properties and have shown that the new probes can overcome the limitations of conventional dyes by demonstrating their usefulness. We first set up the conditions for internal DNA labelling (D. Dziuba et al. *J. Am. Chem. Soc.* **2012**, *134*, 10209) and applied this labelling strategy to study the mechanism of DNA repair (A. A. Kuznetsova et al. *PLoS ONE* **2014**, *9*, e100007).

In a next step, we developed an efficient approach to couple the 3HCs to the pyrimidine bases for external labelling of DNA (D. Dziuba et al. *Chem. Eur. J.* **2014**, *20*, 1998; N. P. F. Barthes et al. *RSC Advances* **2015**, *5*, 33536). The new labels allow discriminating between matched and mismatched dsDNA, as well as between B- and A-forms of DNA/DNA and DNA/RNA duplexes (N. P. F. Barthes et al. *J. Mater. Chem. C* **2016**, *4*, 3010).

The PhD project will focus on the synthesis and study of the 3HCs coupled to the purine bases (A&G). The A and G fluorescent analogues will be incorporated in DNA and used to sense

DNA reparation of A and G damaged bases. We expect through this thesis to complete the fluorescent toolbox having in hand the 4 genetic bases coupled with 3HC. Such tools with dual emission should offer new opportunities to study DNA/protein interactions. The candidate needs to have a strong background in organic chemistry and structural analysis (RMN, MS). Knowledge in chemical biology and nucleoside chemistry would be appreciated. This multidisciplinary project is made in the context of collaboration between our laboratory (UMR 7272 CNRS) and the research group of Dr. N. Kuznetsov (Novosibirsk State University, Russia). Applicants should submit a curriculum vita, a motivation letter and two letters of recommendations to the following e-mail address burger@unice.fr



Prof. Alain BURGER

1) J. Shaya, F. Fontaine-Vive, B. Y. Michel, A. Burger *"Rational design of push-pull fluorene dyes: synthesis and structure-photophysics relationship" Chem. Eur. J.* **2016**, *accepted.*

2) P. F. Barthes, K. Gavvala, D. Dziuba, D. Bonhomme, I. A. Karpenko, A. S. Dabert-Gay, D. Debayle, A. P. Demchenko, R. Benhida, B. Y. Michel, Y. Mély, A. Burger "*Dual emissive analogue of deoxyuridine as a sensitive hydration-reporting probe for discriminating mismatched from matched DNA and DNA/DNA from DNA/RNA duplexes" J. Mater. Chem. C* **2016**, *4*, 3010–3017.

3) N. P. F. Barthes, I. A. Karpenko, D. Dziuba, M. Spadafora, J. Auffret, A. P. Demchenko, Y. Mély, R. Benhida, B. Y. Michel, A. Burger "*Development of environmentally sensitive fluorescent and dual emissive deoxyuridine analogues" RSC Advances* **2015**, *5*, 33536–33545.

4) A. A. Kuznetsova, N. A. Kuznetsov, Y. N. Vorobjev, N. P. F. Barthes, B. Y. Michel, A. Burger, O. S. Fedorova "*New Environment-Sensitive Multichannel DNA Fluorescent Label for Investigation of the Protein-DNA Interactions*", *PLoS ONE* **2014** *9* (6): e100007.

5) D. Dziuba, I. A. Karpenko, N. P. F. Barthes, B. Y. Michel, A. S. Klymchenko, R. Benhida, A. P. Demchenko, Y. Mély, A. Burger "*Rational design of a solvatochromic fluorescent uracil analogue with dual-band ratiometric response based on 3-hydroxychromone" Chem. Eur. J.* **2014**, *20*, 1998–2010.

6) D. Dziuba, V. Y. Postupalenko, M. Spadafora, A. S. Klymchenko, V. Guérineau, Y. Mély, R. Benhida, A. Burger "*A Universal Nucleoside with Strong Two-Band Switchable Fluorescence and Sensitivity to the Environment for Investigating DNA Interaction" J. Am. Chem. Soc.* **2012**, *134*, 10209–10213.

7) C. A. Kenfack, A. S. Klymchenko, G. Duportail, A. Burger, Y. Mély *"Ab initio study of the solvent H-bonding effect on ESIPT reaction and electronic transitions of 3-hydroxychromone derivatives" Phys. Chem. Chem. Phys.* **2012**, *14*, 8910–8918

8) N. Martinet, B. Y. Michel, P. Bertrand, R. Benhida "*Small molecules DNA methyltransferases inhibitors" Med. Chem. Commun.* **2012**, *3*, 263–273.

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