Tracking fluorescence lifetime changes in pH-jump experiments with the Cyan Fluorescent Protein CFP

Anastasia Ioanna Skilitsi[1], Hélène Pasquier[2], Jérémie Léonard[1]

1. Institut de Physique et Chimie des Matériaux de Strasbourg, & Labex NIE CNRS - Université de Strasbourg, Strasbourg, France
2. Laboratoire de Chimie Physique (LCP), Université de Paris Sud, Orsay.

E-mail: Jeremie.Leonard@ipcms.unistra.fr

Recent development of cyan fluorescent proteins (CFPs) follows a step by step evaluation of the spectroscopic effects of crucial mutations within a family of spectrally identical and evolutionary close variants. Through this process it was revealed that a single mutation in the position H148 is adequate to improve the performance of CFPs leading to disappearance of a dimly fluorescent population observed in CFP and also limited pH sensitivity [1]. More generally, CFP and its mutants are model compounds allowing us to decipher the pH-induced conformational changes occurring in fluorescent proteins and their time scale.
Here we report preliminary results of a study aiming at following the fluorescence decay kinetics of CFP and its variant CFP-H148G under out-of-equilibrium initial conditions. At fixed pH (5 or 7.4), both proteins display complex (i.e. multiexponential) fluorescence decay kinetics [2]. We perform pH-jump experiments targeting the determination of the time scale over which the fluorescence decay kinetics of both proteins evolve upon sudden acidification. This in turn reveals the time scale for the protein structural relaxation affecting the chromophore photoreactivity upon pH-jump.
To this end, with a previously validated approach [3], we use droplet microfluidics to produce out-of-equilibrium initial conditions by rapid mixing ($\leq$ 1 ms) within the very small (few 100 pL) volume of water-in-oil droplets. Two inlets of highly concentrated buffer solution at pH=5, one inlet with low concentration buffer solution at pH=7.4 including the protein and a last inlet containing immiscible oil meet at a point called T-junction within the chip. As depicted in the figure a streak camera is used to time resolve the fluorescence decay kinetics of the FPs within the droplets along their propagation. Every fluorescence kinetic trace derives from averaging over a large number of successive identical droplets. Up to 25-30 kinetic traces can be recorded at successive locations corresponding to different propagation times along the microfluidic channel and fitted globally. Preliminary results obtained on CFP indicate that a population exchange between the dominating long-lived fluorescence components 3.8 ns and 2.9 ns occurs on the 240 ms time-scale upon sudden acidification, indicating a structural change of the dominating fluorescent species on the same time scale.

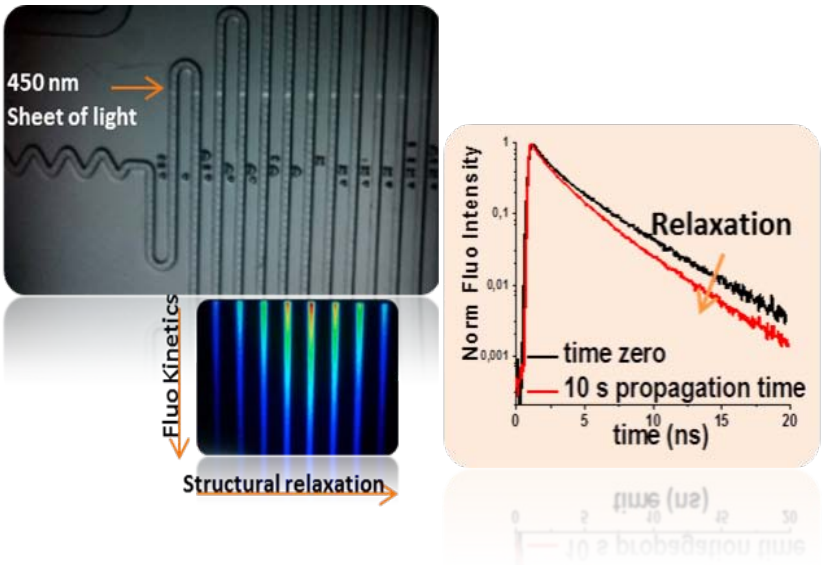

Figure. A microfluidic chip (photograph on the left hand side) is illuminated by a sheet of light formed by a cylindrical lens and focused by an objective. The illuminated section of the chip is imaged along the photocathode wire of a streak camera which acquires 2D data sets (bottom): several fluorescence kinetic traces (false colors; horizontal axis) are acquired simultaneously along the propagation in the channel (vertical axis). Fluorescence kinetic traces are recorded at each of the successive locations of the microfluidic chip (right hand side).

Funding: We acknowledge support from the French Agence Nationale de la Recherche via Grant No. ANR-15-CE11-0006-01 "PICO^2", from the Institut Carnot MICA ("TR-Fluo-Fluidics" project) and from the Région Alsace (Contrat doctoral, No. 493-14-C22).

References:

[1] F. Mérola, M. Erard, A. Fredj and H. Pasquier, *Methods Appl. Fluoresc.*, 2016, 4, 012001

[2] A. Villoing, M. Ridhoir, B. Cinquin, M. Erard, L. Alvarez, G. Vallverdu, P. Pernot, R. Grailhe, F. Merola and H. Pasquier, *Biochemistry (Mosc.)*, **2008**, 47, 12483–12492

[3] S. Maillot, A. Carvalho, J. Vola, C. Boudier, Y. Mély, S. Haacke and J. Léonard, *Lab. Chip*, **2014**, 14, 1767