

INTERNSHIP PROPOSAL

Institute and Group: IBS, group DYNAMOP

Supervisor: Dominique Bourgeois

Phone: 04.57.42.86.44

Email: dominique.bourgeois@ibs.fr

Research project title: Development of fluorescent proteins for quantitative super-resolution microscopy

5 Keywords to describe the project:

Fluorescent proteins; PALM microscopy; photophysics; protein engineering; single molecules

Description of the project (aims, experimental techniques, recommended background):

Super-resolution fluorescence microscopy has become an essential tool to image live biological samples at nanometric resolution. The profound impact that this technique already had on structural and cell biology, and the huge perspectives for further developments have been recognized by the attribution of the chemistry Nobel Prize 2014 to its pioneers. A very popular super-resolution approach is called PALM (PhotoActivated Localization Microscopy). PALM is a single-molecule detection technique, and relies on the use of fascinating fluorescence markers called “phototransformable fluorescent proteins” (PTFPs). PTFPs exhibit amazing photophysical behaviors, for example a green-to-red color change, which are fundamental to the PALM concept. An extension of PALM is qPALM (quantitative PALM), giving access to molecular copy numbers and stoichiometries *in cellulo*. However, current PTFPs are not ideal for qPALM, and they need to be optimized almost for every biological application. At the IBS, we have developed a PALM microscope and we collaborate with teams of biologists, notably in the field of microbiology. In this truly interdisciplinary context, we aim at better understanding PTFPs (down to the atomic scale level), and at engineering improved variants that are optimized for various applications of qPALM. The recruited student, with a background in either physics or structural/cell biology will be involved in the design of new PTFPs.

Relevant publications of the team:

1. Chenxi Duan, Virgile Adam, Martin Byrdin, Jacqueline Ridard, Sylvie Kieffer-Jacquinet, Cécile Morlot, Delphine Arcizet, Isabelle Demachy & Dominique Bourgeois; “Structural Evidence for a Two-Regime Photobleaching Mechanism in a Reversibly Switchable Fluorescent Protein” *J. Am. Chem. Soc.*, (2013), 135, 15841–15850
2. V. Adam, R. Berardozi, M. Byrdin and D. Bourgeois; “Phototransformable fluorescent proteins: Future challenges” *Curr. Opin. Chem. Biol.*, (2014), 92-102.
3. R. Berardozi, V. Adam, A. Martins and D. Bourgeois; “Arginine 66 controls dark-state formation in green-to-red photoconvertible fluorescent proteins”, *J. Am. Chem. Soc.*, (2016) 138 (2), 558–565