



Internship project Master 2 Year 2018-2019

Laboratory: IBS
Team: DYNAMOP/Pixel

Director: Winfried WEISSENHORN
Head of team: Dominique Bourgeois

Name and status of scientist in charge of the project: D. Bourgeois **HDR** yes no

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Title of the project:

Design of advanced fluorescent proteins for super-resolution fluorescence microscopy.

Objectives

Using integrated structural biology and *in cellulo* single-molecule fluorescence imaging, we aim to understand how the performance of fluorescent proteins used as markers in advanced microscopy can be improved, notably at cryogenic temperature.

Abstract

Super-resolution fluorescence microscopy has become an essential tool to image biological samples at nanometric resolution (Chemistry Nobel Prize 2014). 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. However, PTFPs are not ideal, and they need to be optimized almost for every single 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 the various applications. The recruited student will be involved in the design of new PTFPs (low blinking PTFP for quantitative molecular counting, robustly folding PTFP for targeting oxidative cell compartments, PTFP optimized for PALM at cryogenic temperature), and/or possibly in one of the biological application.

Methods

This project stands at the interface between physics, cell biology and structural biology. We associate traditional protein production techniques with advanced structural biology techniques such as kinetic X-ray crystallography and state-of-the-art super-resolution microscopy.

3 relevant publications of the team:

- M. El Khatib, et al, “Rational design of ultrastable and reversibly photoswitchable fluorescent proteins for super-resolution imaging of the bacterial periplasm”, *Scientific Rep.*, (2016) 6, 18459
R. Berardozzi et al, “Arginine 66 controls dark-state formation in green-to-red photoconvertible fluorescent proteins”, *J. Am. Chem. Soc.*, (2016) 138 (2), 558–565
D. Thédié, R. Berardozzi, V. Adam, D. Bourgeois, “Photoswitching of Green mEos2 by Intense 561-nm Light Perturbs Efficient Green-to-Red Photoconversion in Localization Microscopy”, *J. Phys. Chem. Lett.*, (2017), 8, 4424-4430.

Requested domains of expertise

Fluorescence Microscopy, Structural Biology, Biochemistry, Molecular & Cell Biology, Single Molecule

Note: a PhD grant funded by CEA is already available for this project, starting September 2019.