UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2019-2020

Laboratory: IBS
Director: Winfried WEISSENHORN
Team: DYNAMOP/Pixel
Head of team: Dominique Bourgeois

Name and status of scientist in charge of the project: D. Bourgeois **HDR yes** ☒ **no** ☐
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Program of the Master's degree in Biology:

☐ Immunology, Microbiology, Infectious Diseases ☒ Integrative Structural Biology
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology
☐ Planta International

Title of the project:

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

Objectives (up to 3 lines):

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.

Abstract (up to 10 lines):

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 and single-particle tracking, PTFP optimized for PALM at cryogenic temperature), and/or possibly in one of the biological application.

Methods (up to 3 lines):

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.

Up to 3 relevant publications of the team:

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.
E. de Zitter, et al, "Mechanistic investigation of mEos4b suggests a strategy to reduce track interruptions in sptPALM" Nature Meth., in press

Requested domains of expertise (up to 5 keywords):

Biochemistry, molecular & cell biology, interest for single-molecule imaging and /or x-ray crystallography.