noble Alliance for Integrated
Structural & Cell Biology

INTERNSHIP PROPOSAL

Institute and Group: Institut de Biologie Structurale, DYNAMOP/Pixel Group

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Research project title: Development of fluorescent proteins for quantitative and cryogenic 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 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. Two extensions of PALM are qPALM (quantitative PALM), giving access to molecular copy numbers and stoichiometries *in cellulo*, and cryoPALM (PALM at cryogenic temperature) opening the door to better sample preservation and correlative studies with electron microscopy. However, current PTFPs are not ideal for qPALM and cryoPALM, and new variants optimized for these applications are needed. At the IBS, we have developed a PALM microscope and we collaborate with teams of biologists, notably in the field of microbiology. In this interdisciplinary context, we propose two projects. The first one aims at a better understanding of current PTFPs, notably in terms of the dependence of their fluorescence properties on the local nanoenvironment (pH, redox, O2, temperature), with applications in qPALM. The second one aims at rationally engineering improved PTFPs for cryoPALM. To tackle these questions, the recruited student, with a background in either physics or structural/cell biology will use one or several of the following techniques: single-molecule fluorescence imaging, optical spectroscopy, kinetic crystallography.

Justification that the internship's subject fits with the general theme of GRAL:
Super-resolution microscopy has become a central asset for integrated structural cell biology. Developing optimized fluorescent markers for quantitative PALM and cryogenic PALM is thus completely in line with the GRAL program.

Relevant publications of the team:

V. Adam, R. Berardozzi, M. Byrdin and D. Bourgeois; "Phototransformable fluorescent proteins: Future challenges" *Curr. Opin. Chem. Biol.*, (2014), 92-102.

R. Berardozzi, 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

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.