

GRAL PhD PROJECT 2020-2023

Title of the PhD project: Fluorescent proteins to boost single-particle tracking super-resolution microscopy

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Summary of the project: Fluorescence nanoscopy bridges cellular and structural biology. In particular, single-molecule localization techniques such as PALM and STORM provide nanoscale pictures of biological targets in cellulo, either static or dynamic. This project focuses on the dynamic picture, by introducing specific developments of single-particle-tracking approaches based on the use of “phototransformable” fluorescent proteins. Typically, these probes exhibit a highly complex photophysical behaviour: they “blink” and “bleach”, meaning that single-particle tracks are discontinuous and of limited length. The knowledge of fluorescent protein photophysics that we have developed in our team offers new strategies to obtain longer and more continuous tracks, providing more information on the dynamic behaviour of the studied targets, related to e.g. binding, unbinding or changes in diffusion regimes (De Zitter et al, *Nature Meth.*, (2019) 16, 707-710). These developments form the basis of the proposed subject. The work will be carried out in the frame of well-defined collaborative biological projects, including studies of the dynamics of DNA-associated proteins in bacterial cells and of the efferocytosis machinery in mammalian cells.

Keywords: super-resolution microscopy, single-particle tracking, fluorescent markers, protein dynamics, protein-DNA interactions

Applicant profile: Candidates should have a strong background in biophysics and/or biochemistry. Preliminary experience in advanced optical microscopy, protein crystallography or molecular biology will be key advantages.

Three recent publications of the PhD supervisor

E. de Zitter, D. Thédié, V. Mönkemöller, S. Hugelier, J. Beaudouin, V. Adam, M. Byrdin, L. Van Meervelt, P. Dedecker & D. Bourgeois “Mechanistic investigation of mEos4b suggests a strategy to reduce track interruptions in sptPALM”, *Nature Meth.*, (2019) 16, 707-710

K. Floc’h, F. Lacroix, P. Servant, P. Servant, Y.S. Wong, J.P. Kleman, D. Bourgeois & J. Timmins, “Cell morphology and nucleoid dynamics in dividing *Deinococcus radiodurans*”, *Nature Commun.*, (2019), 10, 3815

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 DOI: 10.1021/jacs.5b09923