

Supervisor(s):

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Host laboratory:

Lab : IBS

Host group/team:

I2SR

Title of the M2 research internship:

Design of advanced fluorescent protein markers for super-resolution fluorescence microscopy.

Project summary:

Super-resolution fluorescence microscopy has become an essential tool to image biological samples at the nanoscale. Many super-resolution techniques rely on the use of fascinating fluorescent markers called “photo-transformable fluorescent proteins” (PTFPs). PTFPs exhibit amazing photophysical properties, for example UV-induced irreversible green-to-red color change, or reversible switching between a fluorescent and a nonfluorescent state. However, many mysteries remain about our understanding of how PTFPs work. In addition PTFPs are not ideal fluorophores and need to be optimized almost for every different application. At the IBS, we have developed a comprehensive expertise in the investigation of PTFPs, using a variety of techniques such as UV-vis optical spectroscopy, X-ray crystallography, nuclear magnetic resonance, and single-molecule fluorescence imaging. In this project, we aim at understanding the switching behavior of a red reversibly-switchable fluorescent protein (red RSFP). To date, as opposed to their green counterparts, red RSFPs are not very performant, and ultimately we aim at engineering better variants. The work will rely on the use of state-of-the-art fluorescence microscopy, from sample preparation to image acquisition and analysis. The recruited student should have a background and a strong interest in fluorescence microscopy and biophysics in general.

Keywords:

Fluorescent proteins; super-resolution microscopy; fluorescence imaging ; structural biology

Relevant publications of the team:

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.

V. Adam, et al, “Rational Control of Off-State Heterogeneity in a Photoswitchable Fluorescent Protein Provides Switching Contrast Enhancement”, *ChemPhysChem*, (2022), e202200192

Angela M. R. Mantovanelli, Oleksandr Glushonkov, Virgile Adam, Jip Wulffélé, Daniel Thédié, Martin Byrdin, Ingo Gregor, Oleksii Nevskiy, Jörg Enderlein, and D. Bourgeois*, “Photophysical Studies at Cryogenic Temperature Reveal a Novel Photoswitching Mechanism of rsEGFP2”, *J. Am. Chem. Soc.*, (2023), 145, 14636–14646. Doi: 10.1021/jacs.3c01500

Jip Wulffélé, Arijit Maity, Isabel Ayala, Serge Gambarelli, Bernhard Brutscher* and Dominique Bourgeois*, “Light-Induced Conformational Heterogeneity Induces Positive Photoswitching in Photoconvertible Fluorescent Proteins of the EosFP Family” *J. Am. Chem. Soc.*, (2025), in press. Doi: 10.1021/jacs.4c17311