

**Master 2 internship project
Year 2025-2026**

Laboratory/Institute: IBS
Team: I2SR/Pixel

Director: Winfried WEISSENHORN
Head of the team:

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

- ☐ Microbiology, Infectious Diseases and Immunology ☒ Biochemistry & Structure
☐ Physiology, Epigenetics, Differentiation, Cancer ☐ Neurosciences and Neurobiology

Title of the project:

Deciphering reversible photoswitching in a red fluorescent protein.

Objectives (up to 3 lines):

We study the performance of fluorescent proteins used as markers in super-resolution microscopy. Here, we aim at understanding the switching behavior of a novel reversibly-switchable red fluorescent protein.

Abstract (up to 10 lines):

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 complex switching behavior of a novel reversibly-switchable red 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.

Methods (up to 3 lines):

The work will mainly rely on the use of state-of-the-art fluorescence spectroscopy and imaging, from sample preparation to data acquisition and analysis. The recruited student should have a background and a strong interest in fluorescence techniques and biophysics in general.

Up to 3 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.

Angela M. R. Mantovanelli, Oleksandr Glushonkov, Virgile Adam, Jip Wulffelé, 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 Wulffelé, 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), 147, 12, 10357–10368. Doi: 10.1021/jacs.4c17311

Requested domains of expertise (up to 5 keywords):

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