Supervisor(s):
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Host laboratory:
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Host group/team:
I2SR/PIXEL

Title of the M2 research internship:
Towards quantitative super-resolution fluorescence microscopy.

Project summary:
Super-resolution fluorescence microscopy has become an essential tool to image biological samples at the nanoscale. A popular approach is PALM (PhotoActivated Localization Microscopy). This technique relies on the use of fascinating fluorescent markers called "phototransformable fluorescent proteins" (PTFPs). PTFPs exhibit extraordinary properties, for example a UV-induced green-to-red color change, but their photophysical behavior is also highly complex and poorly understood. Nowadays, PALM imaging aims to be quantitative, e.g. approaches are developed to evaluate the stoichiometry of protein complexes (qPALM) or their diffusion dynamics (sptPALM). To this aim, better PTFPs must be engineered and their photophysical mechanisms must be elucidated. Moreover, for every biological application, experimental conditions and data analysis need to be optimized to take into account the intricate behavior of the employed PTFPs. In this context, the recruited student will be involved on one of the following projects:
(i) Mechanistic understanding of PTFPs by combined NMR and spectroscopic investigations.
(ii) qPALM imaging of the nuclear pore complex (NPC).The NPC has been demonstrated to be an exquisite quantitative reference structure to assess the quality of qPALM images. We aim to image NPCs labeled with various PTFPs through genome editing (CRISPR-Cas9) using qPALM under a variety of experimental conditions, notably at cryogenic temperature. The aim will be to optimize the way qPALM can be conducted.
(iii) Functional dynamics of Src kinases during cell signaling. In the frame of a collaboration with O. Destaing (IAB, Grenoble) we are interested in studying how proteins of the Src family kinase (SFK proteins) reorganize upon various stimuli using an optogenetic approach. We aim to optimize the use of qPALM and sptPALM to follow how the SFK proteins diffuse and cluster within the plasma membrane upon stimulation.
We are looking for a highly motivated student in the field of structural biology, single-molecule fluorescence imaging or data analysis. The student should have a background and a strong interest in biophysics. He/she will be involved in optimizing the data collection and analysis of quantitative PALM data on one of the projects cited above.

Keywords:
super resolution microscopy & fluorescent proteins, NMR & x-ray crystallography & spectroscopy, nuclear pore complex & cell signaling

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.

J. Wulffele, D. Thédié, O. Glushonkov, and D. Bourgeois. "mEos4b Photoconversion Efficiency Depends on Laser Illumination Conditions Used in PALM" J. Phys. Chem. Lett., (2022), 13, 5075–5080.

Angela M. R. Mantovanelli, Oleksandr Glushonkov, Virgile Adam, Jip Wulffelé, Daniel Thédié, Martin Byrdin, Ingo Gregor, Oleksii Nevskyi, 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

Arijit Maity, Jip Wulffelé, Isabel Ayala, Adrien Favier, Virgile Adam, Dominique Bourgeois*, and Bernhard Brutscher* "Structural Heterogeneity in a Phototransformable Fluorescent Protein impacts its Photochemical Properties" Adv. Sci. (2023), 2306272 Doi: 10.1002/advs.202306272