Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

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Title of the M2 research internship:
Study of phototransformable fluorescent proteins photophysics as a function of the local physicochemical environment.

Project summary:
Study of phototransformable fluorescent proteins photophysics as a function of the local physicochemical environment. In the Pixel team at IBS, we study the photophysics of a sub-class of Green Fluorescent Proteins (GFPs), which can change their fluorescence properties under the action of light. These so-called „photo-transformable" fluorescent proteins (PTFPs) play an essential role as fluorescent markers for super-resolved optical microscopy, a key technique for integrated structural cell biology, as applied at the IBS. Our structural studies of PTFPs by crystallography and their functional studies by microscopy are complemented by time resolved studies by optical microspectroscopy – both absorption and fluorescence. Absorption and fluorescence optical spectroscopies provide information on the different electronic states of the photo-switchable entity, the „chromophore", located at the centre of the fluorescent protein beta-barrel scaffold and shielded by it against the bulk solution. On the other hand, subtle movements of this protein cage can have major impact on the chromophore's photochemistry. To be able to observe absorption and fluorescence spectra simultaneously under controlled environmental conditions (such as temperature, solvent viscosity, pH, oxygen level), we have developed the so-called CaI(AI)2doscope – a unique microspectrometer dedicated to the study of fluorescent proteins. At present, the time resolution achieved by the microspectrometer is of the order of a few milliseconds, which is too slow to study some crucial short-lived photophysical states such as the essential triplet state. To access such states, we have recently undertaken an upgrade of the spectrometer, to achieve a time resolution in the sub-millisecond range. The project involves aspects of „technical", „experimental" and „theoretical" nature, such as further improvement of the spectrometer's optical performance and study of the thus accessible short-lived photochemical states as function of temperature, pH, viscosity, oxygen level.

Keywords:
fluorescent proteins, super resolution microscopy, optical spectroscopy

Relevant publications of the team:
Byrdin, M.; Duan, C.; Bourgeois, D.; Brettel, K. A Long-Lived Triplet State Is the Entrance Gateway to Oxidative Photochemistry in Green Fluorescent Proteins. Journal of the American Chemical Society 2018, 140 (8), 2897–2905. https://doi.org/10.1021/jacs.7b12755.

De Zitter, E.; Thédié, D.; Mönkemöller, V.; Hugelier, S.; Beaudouin, J.; Adam, V.; Byrdin, M.; Van Meervelt, L.; Dedecker, P.; Bourgeois, D. Mechanistic Investigation of MEos4b Reveals a Strategy to Reduce Track Interruptions in SptPALM. Nat Methods 2019, 16 (8), 707–710. https://doi.org/10.1038/s41592-019-0462-3.

De Zitter, E.; Ridard, J.; Thédié, D.; Adam, V.; Lévy, B.; Byrdin, M.; Gotthard, G.; Van Meervelt, L.; Dedecker, P.; Demachy, I.; Bourgeois, D. Mechanistic Investigations of Green MEos4b Reveal a Dynamic Long-Lived Dark State. J. Am. Chem. Soc. 2020, 142 (25), 10978–10988. https://doi.org/10.1021/jacs.0c01880.