## GRAL PhD PROJECT 2020-2023

**Title of the PhD project:** Photo-activation mechanism of OCP studied by time-resolved serial crystallography and NMR spectroscopy

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**Summary of the project:** The Orange Carotenoid Protein ($OCP^O$) is a photoactive protein involved in photoprotection of Cyanobacteria. Upon exposure to blue light, the carotenoid pigment undergoes a 12 Å translocation from the interface between the N-terminal (NTD) and the C-terminal (CTD) domains of OCP into the NTD, causing a spectral shift and leading to a separation of the two domains ($OCP^R$). These characteristics open the possibility to rationally engineer OCP variants suited for optogenetic applications and for the regulation of light uptake in artificial photosynthetic systems. However, rational engineering is required to stray from the natural function and its limitations, and to convert OCP into the excellent photoswitch needed for biotechnological purposes. Recently, a photoactivation mechanism was proposed for OCP. We propose a time-resolved serial synchrotron crystallography study on OCP microcrystals complemented by solution NMR spectroscopy coupled with in-situ laser illumination to obtain atomic-resolution information on the light-induced changes in structure and conformational dynamics in the $OCP^R$ state, and to test the proposed photoactivation mechanism). Our results will pave the way to improving the photophysical properties of OCP.

**Keywords:** Orange carotenoid protein, time-resolved serial femtosecond crystallography, solution NMR spectroscopy, artificial photosynthetic systems

**Applicant profile:** We seek to recruit a talented and highly-motivated PhD student trained in biochemistry and structural biology. She/he should be eager to learn new biophysical and structural methods, from serial femtosecond crystallography data processing, structure factor extrapolation, NMR data acquisition and analysis, as well as structure determination. The student will be in charge of sample production during the first year of the PhD, which should allow him to obtain enough sample for his thesis.

**Recent publications:**

Chromophore twisting in the excited state of a photoswitchable fluorescent protein captured by time-resolved serial femtosecond crystallography. Coquelle N, …, *Colletier JP, *Schlichting I, *Weik M. Nat Chem. 2018 Jan;10(1):3137.
De novo phasing with X-ray laser reveals mosquito larvicide BinAB structure. *Colletier JP, …, *Eisenberg DS. Nature. 2016 Nov 3;539(7627):43-47.
NMR reveals light-induced changes in the dynamics of a photoswitchable fluorescent protein. N. E. Christou, I. Ayala, K. Giandoreggio-Barranco, M. Byrdin, V. Adam, D. Bourgeois, and B. Brutscher, Biophys. J. (2019), 117: 2087–2100.