Master 2 internship project, **Year 2019-2020**

Institute: Institute of Structural Biology
Director: Winfried Weissenhorn

Team: Serial Nano Crystallography (SNaX)
Head of the team: Jacques-Ph. Colletier

Name and status of the scientist in charge of the project: Jacques-Ph. Colletier (DR2)
HDR: Yes (EDCSV)

Address: 71 avenue des Martyrs, Grenoble 38044 Cedex 9
Phone: +33 457 428 515 **e-mail:** colletier@ibs.fr

Program of the Master's degree in Biology:

☐ Immunology, Microbiology, Infectious Diseases
☐ Physiology, Epigenetics, Differentiation, Cancer
X Integrative Structural Biology
☐ Neurosciences and Neurobiology
X Planta International

Title of the project: Insights into the photo-activation mechanism of the Orange Carotenoid Protein (OCP) by time-resolved serial femtosecond crystallography (TR-SFX) and nuclear magnetic resonance (NMR) spectroscopy.

Objectives (up to 3 lines): Our goal is to obtain atomic-resolution insights into the photoactivation mechanism of OCP, with view to rationally design variants suited for biotechnological applications such as artificial photosynthetic systems and optogenetics.

Abstract (up to 10 lines):

To protect themselves from intense light energy damage, cyanobacteria synthesize a two-domain (N-terminal (NTD) and C-terminal (CTD) domains) photoactive soluble protein, OCP, capable of dissipating the excessive energy arriving to their light harvesting antennas . Upon photon absorption, the protein transitions from a compact orange "non-active" state (OCP°) to an extended red "active" state (OCP^{r}), with migration of the carotenoid pigment from the interface between the two domains into the NTD. It remains poorly understood how the energy absorbed by the carotenoid is funneled into the protein scaffold, fueling for the structural transition associated with photoactivation. We propose to use a combination of two structural methods, TR-SFX and NMR, with view to illuminate the fs-s photo-triggered and equilibrium structural dynamics of OCP and *in fine* design more potent OCP variants.

Methods (up to 3 lines):

Bacterial transfection and culture, protein purification and crystal production, biophysical methods for protein characterization, time-resolved serial crystallography at synchrotrons and X-ray free electron lasers (XFEL), isotopic labelling, NMR data acquisition and analysis.

Up to 3 relevant publications of the team:

1. Cyanobacterial photoprotection by the orange carotenoid protein. Kirilovsky & Kerfeld, 2016, *Nat. Plants,* 2:16180

2. De novo phasing with X-ray laser reveals mosquito larvicide BinAB structure. Colletier *et al.*, 2016, *Nature,* 536:43

3. Chromophore twisting in the excited state of a photoswitchable fluorescent protein captured by time-resolved serial femtoseconf crystallography. Coquelle *et al.*, 2018, Nat. Chem. 10:31

Requested domains of expertise (up to 5 keywords):

Interests on the field of Biochemistry, Crystallography, NMR data acquisition, Biophysical characterization of proteins, Microbiology