UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Internship project Master 2
Year 2018-2019

Laboratory/Institute: Institute of Structural Biology
Director: Winfried Weissenhorn
Team: Dynamics and kinetics of molecular processes
Head of the team: Martin Weik

Name and status of the scientist in charge of the project:
Elena Andreeva (*Ph.D.*) / Jacques-Philippe Colletier (*DR*)
HDR: yes ☐ no ☒
Address: 71 Avenue des Martyrs, 38044 Grenoble Cedex 9
Phone: 04.57.42.87.37 **e-mail:** eandreeva@ibs.fr

Program of the Master's degree in Biology:

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

Title of the project:

In-vivo crystallization of Orange Carotenoid Protein (OCP) in *Bacillus thuringiensis* (Bt).

Objectives (up to 3 lines):

The OCP is a 35 kDa carotenoid binding soluble protein involved in the photoprotection mechanism of cyanobacteria. In this project we aim to obtain *in-vivo* grown OCP crystals by recombinant crystallization in Bt cells. Our second goal is to obtain a carotenoids expression in Bt, needed to have a functional OCP protein.

Abstract (up to 10 lines):
Intense energy is harmful for all photosynthetic organisms, such as cyanobacteria. In order to protect themselves, these bacteria synthesize a photoactive soluble Orange Carotenoid Protein (OCP) who dissipate the excess of energy and quench the produced singlet oxygen. Upon blue-green light illumination, the protein undergoes two distinct states: an orange "non-active" one (**OCP°**) and a red "active" state (**OCP^{r})** known to be essential for the photoprotective mechanism. While recent studies showed that the dynamics between the two states is on the nanosecond to second timescale, it is still unknown how the energy absorbed by the carotenoid triggers the activation of **OCP^{r}** state. To elucidate this mechanism, in our group we use a new method that allows collecting structural data on nanocrystals on the fs time scale, such as **TR** serial femtosecond crystallography (**SFX**) using X-ray free electron lasers (**XFELS**). Thus, our main goal will be to obtain *holo*-OCP stable nanocystals *in-vivo* by recombinant expression in *Bacillus thuringiensis*. We will also engineer the recombinant production of carotenoids in Bt cells in order to establish functional OCP and have a better understanding of the structural changes that accompany OCP photoactivation.

Methods (up to 3 lines):

Design and construction of shuttle vectors, bacterial transfection and culture, protein purification and crystal production, time-resolved serial crystallography at synchrotrons and X-ray free electron lasers (XFEL), data analysis

Up to 3 relevant publications of the team:

1. Cyanobacterial photoprotection by the orange carotenoid protein. Kirilovsky, D. et al., *Nat.Plants* 2016: 2, 16180

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

3. Chromophore twisting in the excited state of a photoswitchable fluorescent protein captured by time-resolved serial femtoseconf crystallography. Coquelle, N. et al., Nat. Chem. 2017,doi:10.1038/nchem.2853

Requested domains of expertise (up to 5 keywords):

Interest in Structural Biology, Biochemistry, Biophysical characterization of proteins, Microbiology