UFR de Chimie et de Biologie
UNIVERSITÉ Grenoble Alpes

Master's degree in Biology – Chemistry-Biology Department

Master 2 internship project
Year 2019-2020

Laboratory/Institute: Institut de Biologie Structurale
Director: Winfried Weissenhorn
Team: SNaX
Head of the team: Jacques-Ph. Colletier

Name and status of the scientist in charge of the project: Guillaume Tetreau
HDR: yes ☐ **no** X
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Program of the Master's degree in Biology:

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

Title of the project: *In vivo crystallization as a novel strategy to obtain atomic-resolution insights into protein structure and dynamics.*

Objectives (up to 3 lines):

The goal of this project is to develop a new *in vivo* crystallization approach based on the crystalliferous bacterium *Bacillus thuringiensis.* The student will be associated to all steps required to attain and publish a proof-of-concept, including production, purification and characterization of the recombinant nanocrystals.

Abstract (up to 10 lines):

The advent of X-ray free electron lasers (XFELs) has revolutionized structural biology, opening doors to time-resolved experiments at ultra-fast time-scale (fs-ps). A pre-requisite is yet to produce small and calibrated diffraction-grade crystals, as required to reach full activation of crystalline proteins in a time-resolved experiment. The crystals will also have to be produced in large amount, as required by the serial data collection approach whereby each crystal is only exposed once. Our lab recently demonstrated that diffraction-grade calibrated nanocrystals can be obtained by recombinant expression in the crystalliferous bacterium *Bacillus thuringiensis* and that a structure can be determined *de novo* from data collected on these crystals (Colletier et al., 2016, Nature; Sawaya et al., 2014, PNAS). We here propose to build on this to develop a new *in vivo* nano-crystallization approach whereby *B. thuringiensis* and its naturally crystalline Cry/Cyt proteins are used as crystallization platform and chaperones, respectively. Beamtime has already been secured to evaluate recombinant nanocrystals qualities at the European XFEL.

Methods (up to 3 lines):

The project will involve shuffle-plasmid construction, cloning, amplification and expression of these in both *E. coli* and *Bt*, recombinant nano-crystals production in *Bt*, and crystal purification and quality assessment by SDS-PAGE, Western Blot, SEM, AFM and diffraction at the ESRF and the European XFEL.

Up to 3 relevant publications of the team:

Grünbein ML. *et al.* (2018) Megahertz data collection from protein microcrystals at an X-ray free-electron laser. Nature Communication 9(1): 3487.

Colletier JP. *et al.* (2016) De novo phasing with X-ray laser reveals mosquito larvicide BinAB structure. Nature 539(7627): 43-47.

Sawaya MR. *et al.* (2014) Protein crystal structure obtained at 2.9 Å resolution from injecting bacterial cells into an X-ray free-electron laser beam. PNAS 111(35): 12769-74

Requested domains of expertise (up to 5 keywords):

Molecular biology; bacteriology; biochemistry;