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:
Guillaume Tetreau (Post-Doctoral Scientist) / Jacques-Philippe Colletier (DR)
HDR: yes ☐ no ☒
Address: 71 Avenue des Martyrs
Phone: **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:

Characterization of efflux pumps involved in antibiotic resistance in the human pathogen *Providencia stuartii*.

Objectives (up to 3 lines):

The aim of the project is to produce in *E. coli* different subunits of RND-type efflux pumps of *P. stuartii*, to purify and crystallize them in order to obtain diffraction data at Synchrotron and solve their structure to understand their function.

Abstract (up to 10 lines):
Efflux pumps are transmembrane protein complexes that allows the transportation of toxic molecules (metabolites and/or antibiotics) across the membrane, from the cytoplasm to the outside of the cell. In Gram-negative bacteria, RND-type efflux pumps are constituted of three sub-units: AcrB (inner membrane), AcrA (periplasm) and TolC (outer membrane). *Providencia stuartii* is an opportunistic human pathogen, responsible for deleterious urinary infections. Due to its naturally high level of resistance to a wide range of antibiotics and to its capacity to form adherent biofilms, infections to *P. stuartii* can have detrimental effects on patient's health. Therefore, understanding how *P. stuartii* resist to different classes of antibiotics is of high importance. By genome mining and quantitative PCR analysis, we already identified two genes for each sub-units that are differentially expressed upon exposure to different antibiotics. Now, we aim at characterizing these sub-units and understanding their involvement in the resistance phenotype.

Methods (up to 3 lines):

Plasmid construction; PCR; Protein production in *E. coli*; Protein purification; Quality checking; Crystallization; Synchrotron data analysis.

Up to 3 relevant publications of the team:

1. El-Khatib (2018) Porin self-association enables cell-to-cell contact in *Providencia stuartii* floating communities. PNAS 115(10): E2220-2228.

2. El-Khatib (2017) Porin self-association enables cell-to-cell contact in *Providencia stuartii* floating communities. PLoS ONE 12(3): e0174213.

3. Song (2015) Understanding Voltage Gating of *Providencia stuartii* Porins at Atomic Level. PLoS Comput. Biol. 11(5): e1004255.

Requested domains of expertise (up to 5 keywords):

Molecular biology; Microbiology; Biochemistry; Crystallography