Masters internship project in Marseille at the microbiology/biochemistry/biophysics interface

Academic year 2020-2021

Title of the project: Structural characterization of the iron-sulfur cluster-containing PAS domain of OrpR from *Desulfovibrio vulgaris* Hildenborough.

Location: Marseille (France), Mediterranean Institute of Microbiology http://www.imm.cnrs.fr/. The Mediterranean Institute of Microbiology (IMM) is one of the largest French research institutes for microbiology gathering about 300 people, including 140 permanent positions and 4 research laboratories, and associating 14 research and technical platforms. The institute offers a multidisciplinary, international, and vibrant environment for science research in microbiology.

Research laboratories (Aix-Marseille University & CNRS): Laboratoire de Chimie Bactérienne (LCB), Laboratoire de Bioénergétique et Ingénierie des Protéines (BIP) et Laboratoire Information Génomique et Structurale (IGS), Marseille, France. All three laboratories are located nearby and belong to IMM.

Supervision Teams: This interdisciplinary internship will be located primarily on the CNRS Campus (31 chemin J. Aiguier, 13402 Marseille) in the LCB (Team Fe-S clusters biogenesis and homeostasis) and BIP labs (Team Biophysics of Metalloprotein), with some experiments performed in the IGS lab (university campus of Luminy).

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Project summary:

Strict anaerobes living in anoxic habitats can be exposed to various environmental stresses. Consequently, they must rapidly detect these changes and adapt their metabolism to varying conditions. In the strict anaerobe Deltaproteobacteria *Desulfovibrio vulgaris* Hildenborough, we have identified a σ^{54}-dépendent transcription factor that plays a crucial role in the regulation of the protein complex ORP, which is linked to anaerobic life (Fievet, 2011 and 2014). This transcription factor, called OrpR, is the first known redox regulatory protein containing an iron-sulfur cluster isolated from anaerobes. It is composed of three domains from N- to C-terminal: a regulatory domain (PAS), a central ATPase domain (AAA^+), and a DNA-binding domain (HTH) (Fig. 1).

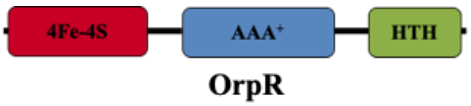

Figure 1: Schematic representation of the iron-sulfur-containing transcriptional regulator OrpR, which is involved in detecting redox stress in the environment of the anaerobe *Desulfovibrio vulgaris* Hildenborough

We have recently proposed that the regulatory PAS domain contains a [4Fe4S] cluster that is sensitive to redox potential variations in the cell (Fievet et al, submitted). The overall goal of this project is to

elucidate the molecular regulatory mechanisms of this novel transcriptional regulator in varying redox conditions, by using an interdisciplinary approach combining microbiology, biochemistry, and biophysics. The first step towards this goal will be the structural characterization of the Fe-S cluster-containing PAS domain of OrpR.

First, bioinformatics tools will be used to design several truncated constructs of the OrpR PAS domain. Full-length OrpR and shorter PAS-domain constructs will be overproduced in *Escherichia coli* and purified in anaerobic conditions. Various biochemical and biophysical techniques (size-exclusion chromatography, microscopy, dynamic light scattering, circular dichroism …) will be used to (i) determine their stability, oligomerization state, and homogeneity; (ii) determine optimal conditions for their crystallization, and (iii) setup crystallization conditions in anaerobic atmosphere. In parallel, various analytical and spectroscopic approaches (protein and elemental content, UV-Vis, CD and EPR) will allow characterization of the iron-sulfur cluster (stoichiometry, reduction potential, affinity) in the various constructs. Results from this project will constitute an essential first step toward understanding the molecular mechanisms by which OrpR can sense and respond to redox stresses in anaerobes.

Références

Fiévet A, My, L, Cascales E, Ansaldi M, Pauleta SR, Moura I, Dermoun Z, Bernard CS, Dolla A and Aubert C. *J. Bacteriol.*, **2011**, 193, 3207-3219.

Fiévet A, Cascales E, Valette O, Dolla A, Aubert C. *PLoS One*, **2014**, 9(1): e86507

Fiévet A, Merrouch, M, Brasseur, G., Eve, D., Biondi, E., Valette, O. Pauleta, S., Dolla A, Dermoun, Z., Burlat, B., Aubert C. *submitted.*

Candidate profile: The candidate should have an education background in Biochemistry, Structural Biology and/or Chemistry for Biological Sciences, with strong interest in experimental work in an interdisciplinary team. The start date and duration of the internship will be adjusted according to the candidate's availability, the academic requirements of the candidate's University and French regulation. French language is not mandatory.

Salary: This internship will be funded by the Institute "Microbiologie Bioénergies et Biotechnologie" - IM2B (AMX-19-IET-006), Programme Investissements d'Avenir, Initiative d'Excellence d'Aix-Marseille Université A*MIDEX. Institute IM2B is a network of internationally recognised laboratories, to strengthen interdisciplinary research and education in the field of microbiology and its applications in Bioenergy, Environment and Health.

The training net salary is about 540 euros/month. The selected candidate will be encouraged to apply for an Erasmus exchange Program for additional financial support.

Application process: Interested candidates should first contact the supervision's Team by email and include (a) a cover letter explaining their motivation for the internship's project and relevance of their academic career, (b) a CV (in French or English) including their grades. Recommendation letters will be requested later for selected applicants.