Internship project Master 2
Year 2017-2018

Laboratory/Institute: Institut de Biologie Structurale **Director:** Winfried Weissenhorn
Team: Metalloproteins **Head of the team:** Yvain Nicolet

Name and status of the scientist in charge of the project: Juan Fontecilla-Camps **HDR:** **yes** x
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Program the Master's degree in Biology:

☐ Neurosciences and Neurobiology ☐ Immunology, Microbiology, Infectious Diseases

x Integrative Structural Biology ☐ Physiology, Epigenetics, Development, Differentiation

Title of the project:

Control of gene expression by gas-sensing iron-sulfur cluster proteins

Objectives (up to 3 lines):

To understand at the atomic level: (1) how a protein-bound iron-sulfur cluster reacts with molecular oxygen, in the case of the Fumarate Nitrate Reduction regulator (FNR) sensor, or nitric oxide, in the case of the NO sensitive response regulator NsrR, and (2) how that reaction changes the sensors DNA binding properties.

Abstract (up to 10 lines):

Facultative anaerobic bacteria, including several pathogens, are capable of using the reactivity of protein bound iron-sulfur (FeS) clusters toward gases like O_2 and NO for the regulation of gene expression.We have already reported several O_2-induced [4Fe-4S] cluster degradation products in an O_2-sensitive bacterial enzyme [1]. We have also been able to crystallize and solve the first structures of the O_2 sensor FNR [2], the global regulator of the transition between aerobic and anaerobic metabolism in bacteria, and the NO sensor NsrR [3], which allows pathogens to defend themselves against NO produced by human lymphocytes. The M2 project will consists of producing and characterizing, both functionally and structurally, several variants of both proteins in order to elucidate the atomic details of the gas reactions with the FeS cluster and the resulting structural changes that modulate DNA binding activity. We are well equipped with a series of anaerobic glove boxes that will allow the student to perform all the required experiments.

Methods (up to 3 lines):

Anaerobic expression, purification, characterization and crystallization of FNR and NsrR variants under *controlled* O_2 and NO concentrations inside glove boxes, including DNA binding studies, followed by X-ray crystallographic analyses (see also our web site: http://www.ibs.fr/groupes/groupe-metalloproteines/).

Up to 3 relevant publications of the team:

[1] Y Nicolet, R Rohac, L Martin and JC Fontecilla-Camps. X-ray snapshots of possible intermediates in the time course of synthesis and degradation of protein-bound Fe_4S_4 clusters. *Proc Natl Acad Sc USA* 2013, **110**, 7188-7192.
[2] A Volbeda, C Darnault, O Renoux, Y Nicolet & JC Fontecilla-Camps. The crystal structure of the global anaerobic transcriptional regulator FNR explains its extremely fine-tuned monomer-dimer equilibrium. *Science Advances* 2015, **1**(11), e1501086.
[3] A Volbeda, EL Dodd, C Darnault, JC Crack, O Renoux, MI Hutching, NE Le Brun & JC Fontecilla-Camps: Crystal structures of the NO sensor NsrR reveal how its iron-sulfur cluster modulates DNA binding. *Nature Communications* 2017, **8**, 15052.

Requested domains of expertise (up to 5 keywords):

Chemistry, biochemistry, structural biology, notions of molecular biology