

**Internship project Master 2
Year 2018-2019**

Laboratory/Institute: Institut de Biologie Structurale **Director:** Weissenhorn, Winfried
Team: Goupe métalloprotéines **Head of the team:** Yvain Nicolet
Name and status of the scientist in charge of the project: Eve de Rosny - **HDR:** yes
Address: 71 avenue des Martyrs, CS 10090, 38044 Grenoble Cedex 9
Phone: 04 57 42 85 17 **e-mail:** eve.derosny@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:

Structural approach of oxygen sensing by the fumarate and nitrate reduction regulator FNR.

Objectives:

Investigation of the molecular mechanism leading to FNR monomerization upon O₂ exposure, using biochemical and biophysical techniques.

Abstract:

The FNR is a transcription factor that coordinates the switch between aerobic and anaerobic metabolism in facultative anaerobic bacteria. It contains a N-terminal domain with an iron-sulfur cluster that detects the presence of O₂ and a C-terminal DNA-binding domain that recognizes specific DNA binding sequences within target promoters. Under anaerobic conditions, FNR is a [4Fe-4S]²⁺ protein that forms dimers. Under aerobic conditions the cluster is rapidly degraded which leads to monomerization and loss of DNA binding. The first X-ray structure of the FNR was solved in our laboratory. Subtle analysis suggests that the monomerization involves an “unzipping” process that starts very locally by the dissociation of two symmetry-related salt bridges and propagates along the dimer interface (see ref below). The Master 2 project aims to validate this hypothesis by studying specific mutants of the FNR that are involved in the monomerization-dimerization process.

Methods:

Directed mutagenesis - Protein expression and purification - Analytical gel chromatography – Determination of melting temperatures using intrinsic fluorescence.

Most of the experiments will be performed under anaerobic conditions (glove boxes)

3 relevant publications of the team:

Volbeda A, Dodd EL, Darnault C, Crack JC, Renoux O, Hutchings MI, Le Brun NE, Fontecilla-Camps JC (2017) “Crystal structures of the NO sensor NsrR reveal how its iron-sulfur cluster modulates DNA binding”. *Nat Commun* 8:15052. DOI: 10.1038/ncomms15052

Pérard J, Coves J, Castellan M, Solard C, Savard M, Miras R, Galop S, Signor L, Crouzy S, Michaud-Soret I, de Rosny E. (2016), “Quaternary structure of Fur proteins, new subfamily of tetrameric proteins”. *Biochemistry*, 55, 1503–1515

Volbeda A, Darnault C, Renoux O, Nicolet Y, Fontecilla-Camps JC (2015), “The crystal structure of the global anaerobic transcriptional regulator FNR explains its extremely fine-tuned monomer-dimer equilibrium”. *Sci Adv* 1:e1501086. DOI: 10.1126/sciadv.1501086

Requested domains of expertise:

Good knowledge and interest in biochemistry and molecular biology.