Grenoble Alliance for Integrated Structural & Cell Biology

INTERNSHIP PROPOSAL

Institute and Group: Institut de Biologie Structurale – Metalloproteins group

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Research project title:

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

5 Keywords to describe the project:

Transcription factors, oxygen sensing, iron-sulfur cluster, glove boxes, structural and biophysical characterizations.

Description of the project (aims, experimental techniques, recommended background):
Many bacteria, such as *E. coli*, can grow either in anaerobic or aerobic environments. FNR is the transcription factor that coordinates the switch between aerobic and anaerobic metabolism. It contains an N-terminal domain with an iron-sulfur cluster that detects the presence of O_2 and a C-terminal DNA-binding domain that recognizes specific DNA binding sequences within target promoters. Under anaerobic conditions, FNR forms dimers, in the presence of O_2 its $[4Fe\text{-}4S]^{2+}$ cluster is rapidly degraded which leads to monomerization and loss of DNA binding. The first X-ray structure of FNR was recently solved in our laboratory. Our 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 1). The Master 2 project aims to validate this hypothesis by studying mutants of FNR, changing residues that are involved in the monomerization-dimerization process. The main methods used will be: site directed mutagenesis, protein purification, size exclusion chromatography, spectrofluorometry and X-ray crystallography. Most of the experiments will be performed under anaerobic conditions (glove boxes). The candidate should have good knowledge and interest in biochemistry and molecular biology.

Justification that the internship's subject fits with the general theme of GRAL:
This project aims to understand the modulation of the biological function of the FNR using structural and biophysical approaches. The first step involves *in vitro* characterizations of functionally altered FNR variants that would next be generated in a cellular context.

Relevant publications of the team :

1. 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
2. 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
3. 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