Master 2 research internship in Integrated Structural & Cell Biology in Grenoble

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Title of the M2 research internship:
Structural approach to NO sensing at the [4Fe-4S] cluster of the transcriptional regulator NsrR

Project summary:
All microorganisms use transcription factors (TFs) to rapidly sense and respond to environmental changes. In the Metalloproteins unit, we are interested in members of the CRP-FNR and Rrf2 families of dimeric TFs that can use the redox properties, intrinsic lability and gas-binding affinity of iron-sulfur clusters to modulate their DNA binding. The well-studied Fumarate Nitrate Reductase regulator (FNR) uses a [4Fe-4S] cluster to sense O2 levels and to regulate a dimer monomer transition mediated by cluster disassembly and a metastable protein interface. Only the dimer binds to specific DNA sites. Conversely, regulation of DNA binding capabilities of the Rrf2 family of TFs depends on protein conformational changes caused by the status of the cluster or its absence. The Rrf2 member [4Fe-4S]-NsrR from Streptomyces coelicolor modulates expression of three different promoters via complex formation with NO, thus orchestrating the cellular response leading to detoxification of this gas. The well-established differential binding of NsrR to those promoters is modulated by the degree of NO-induced modification of its [4Fe-4S] cluster. The internship research will consist of finding conditions where NO-[4Fe-4S]-NsrR complexes with different NO gas-to-cluster ratios are stable enough to be characterized by biophysical methods including UV-vis spectroscopy, non-denaturing mass spectrometry and X-ray crystallography. Whenever possible, these complexes will be combined with their cognate DNA and the stability of the resulting species will be also monitored and structurally characterized. Previous work by our British collaborators (Prof. Nick Le Brun, University of East Anglia, Norwich) has hinted at the existence of intermediates with rather long half-lives. These conditions need to be studied in greater detail, especially concerning the dependence of the NO-NsrR stoichiometry on experimental temperature, pH and ionic strength. This work will be carried out using our anaerobic setup of six gloves boxes dedicated to express, purify and characterize metalloproteins.

Keywords:
iron-sulfur cluster sensor, DNA binding specificity, adaptation of bacteria to NO stress

Relevant publications of the team:
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.

The crystal structure of the global anaerobic transcriptional regulator FNR explains its extremely fine-tuned monomer-dimer equilibrium. Volbeda A, Darnault C, Renoux O, Nicolet Y, Fontecilla-Camps JC. Sci Adv. 2015 Dec 4;1(11):e1501086. doi: 10.1126/sciadv.1501086.

Crystal structures of the NO sensor NsrR reveal how its iron-sulfur cluster modulates DNA binding. Volbeda A, Dodd EL, Darnault C, Crack JC, Renoux O, Hutchings MI, Le Brun NE, Fontecilla-Camps JC. Nat Commun. 2017 Apr 20;8:15052. doi: 10.1038/ncomms15052.

Crystal Structure of the Transcription Regulator RsrR Reveals a [2Fe-2S] Cluster Coordinated by Cys, Glu, and His Residues. Volbeda A, Martinez MTP, Crack JC, Amara P, Gigarel O, Munnoch JT, Hutchings MI, Darnault C, Le Brun NE, Fontecilla-Camps JC. J Am Chem Soc. 2019 Feb 13;141(6):2367-2375. doi: 10.1021/jacs.8b10823.

Electron and Proton Transfers Modulate DNA Binding by the Transcription Regulator RsrR. Crack JC, Amara P, Volbeda A, Mouesca JM, Rohac R, Pellicer Martinez MT, Huang CY, Gigarel O, Rinaldi C, Le Brun NE, Fontecilla-Camps JC. J Am Chem Soc. 2020 Mar 18;142(11):5104-5116. doi: 10.1021/jacs.9b12250.