**Proposition de sujet de thèse**

**Campagne 2017 d’attribution des contrats doctoraux attribués à EDCSV**

**Directeur de thèse** (Supervisor), HDR rattaché à EDCSV:

Volbeda Anne, Institut de Biologie Structurale(IBS), 71 avenue des Martyrs Grenoble

Année d’obtention de l’HDR : 2002

**Co-Directeur de thèse** (éventuel), titulaire HDR :

 *Nom, Prénom, coordonnées, année d’obtention de l’HDR*

**Ou Co-encadrant** (si non HDR) :

Cherrier Mickaël V., Institut de Biologie Structurale (IBS)

*En cas de co-direction ou co-encadrement,* ***les taux d’encadrement seront de 50% chacun****.*

**Unité de Recherche**/Laboratory :

 Winfried Weissenhorn *(IBS)*

**Equipe de recherche**/Research team :

 Yvain Nicolet *(groupe métalloprotéines)*

**Titre du projet de thèse** (en français):

Etude structurale de métalloprotéines par microscopie électronique en condition anaérobie : l’hydrogénase à [Fe-Fe] multimérique de *Desulfovibrio fructosovarans*.

**Titre du projet de thèse** (en anglais):

Structural study of metalloproteins in anaerobic conditions using electron microscopy: the multimeric [Fe-Fe] hydrogenase from *Desulfovibrio fructosovarans*.

**Résumé** (en anglais): Précisez le contexte, les objectifs, les méthodes (500 mots maximum)

About one quarter to one third of the known proteins have in their structure a transition metal. Thanks to this they can catalyze a larger variety of reactions than the ones accessible with only the twenty amino acids. So, many metalloproteins are involved in key cell biological processes, such as photosynthesis, respiration, or oxygen transport. The most common metal atom found in proteins is iron, often present in an iron-sulfur cluster [Fe-S]. Such clusters are involved in different processes such as electron transfer, enzymatic catalysis, and a sensor of environmental or intracellular conditions. But [Fe-S] clusters are also often sensitive to degradation by oxygen. As a consequence, the structural study of FeS metalloproteins requires to work in a strict anaerobic environment.

Such studies are at present mainly performed using X-ray crystallography. However, the structural study of the different complexes that a protein can form with its partners is limited by the difficulty to grow diffracting crystals. When using cryo-electron microscopy (cryoEM) instead, the protein complexes are directly visualized, without the need to have crystals. During the last few years, electron microscopy has become a more and more powerful technique and it is now common to visualize details up to 3 Å resolution. In the case of metalloproteins complexes, the limitation for the use of electron microscopy techniques is their sensitivity to oxidation by oxygen. As a consequence, up to now only few structures of metalloproteines sensitive to oxygen were resolved using cryoEM.

The goal of this PhD proposal is to develop, with the candidate, the tools necessary to study by cryoEM macromolecular assemblies possessing metallic clusters sensitive to oxidation by oxygen. The candidate will study a multimeric [Fe-Fe] hydrogenase from the sulfate reducing bacterium *Desulfovibrio fructosovorans*. Its active site, also called the *H cluster*, consists of a [2Fe] subcluster, containing CN and CO groups, which is bridged by a single cysteine residue to a [4Fe-4S] cluster. As all hydrogenases, it catalyzes the reversible oxidation of molecular hydrogen: H2 ⬄ 2 H+ + 2 e-. The *D. fructosovorans* hydrogenase has the specificity to use two different acceptors for the electrons generated by this reaction: NADP and ferredoxin. In addition to the fundamental interest of the structural study of this protein, for which no structure is known yet, a better understanding of the reaction catalyzed could help the development of new processes for biological hydrogen production.

In the near future, we also plan to use the same technology to study many other complexes of metalloproteins in anaerobic conditions. We are especially interested in:

* The maturation complex of the nitrogenase active site, a key enzyme of the nitrogen cycle, which transforms N2 into bio-accessible ammonia (NH3).
* The mitochondrial ISC proteins involved in the biogenesis of [Fe-S] in mammals, involved in a significant number of diseases.

The candidate will work in a dynamic environment involving the utilization of many different techniques, which should be useful for his/her future scientific career.

**Mots-clés** (5 maximum) : en anglais et en français

Metalloprotéines ; Biologie Structurale ; Complexes protéiques ; microscopie électronique ; Hydrogénase

Metalloproteins ; Structural Biology ; Proteins complexes ; electron microscopy ; hydrogenase

**Sujet éligible à une allocation de la Fondation pour la recherche médicale (FRM) :**

Oui **□** Non **X**

**Profil du candidat souhaité :**

**Le/la candidat(e) devra avoir de bonnes connaissances en biochimie et en biologie structurale. Il/elle devra travailler dans un environnement pluridisciplinaire à l’interface de la biologie, la biophysique et la chimie. Des connaissances en chimie et/ou en informatique seraient un plus.**

**Trois publications récentes du Directeur de thèse** (et du co-directeur, s’il y a lieu) :

**A Volbeda, L Martin, E Barbier, O Gutiérrez-Sanz, AL De Lacey, P-P Liebgott, S Dementin, M Rousset & JC Fontecilla-Camps: Crystallographic studies of [NiFe]-hydrogenase mutants: towards consensus structures for the elusive unready states. J. Biol. Inorg. Chem. (2015) 20, 11-22**

**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.**

**A Volbeda, C Darnault, D Reichmann, O Renoux, S Ollagnier de Choudens & JC Fontecilla-Camps: Crystal structures of quinolinate synthase in complex with a substrate analogue, the condensation intermediate and substrate-derived product. J Am Chem Soc (2016), 138, 11802-9**

**Docteurs encadrés par le Directeur de thèse** (et du co-directeur s’il y a lieu) **ayant soutenu leur thèse** (dans les 5 dernières années). Indiquer la date de soutenance, la durée de la thèse (en mois), les publications relatives au sujet de thèse et leur situation actuelle :

**Thèses en cours encadrées par le Directeur de** **thèse** (et du co-directeur s’il y a lieu, dupliquer le tableau)

|  |  |  |  |
| --- | --- | --- | --- |
| Nom, Prénom du doctorant | Date de début de thèse | Type de financement | Indiquer, le cas échéant, s’il s’agit d’une co-direction, d’une co-tutelle,… |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

**Nombre de chercheurs et enseignant-chercheurs titulaires d’une HDR dans l’équipe** : **4**

**Nombre total de thèses en cours dans l’équipe : 2**