Sujet de stage de Master 2 (

Laboratoires : Modélisation et Exploration des Matériaux (MEM) / laboratoire de chimie et

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Titre du sujet: Investigation of the peculiar nickel-binding site of the metalloprotein CooT by hyperpolarized solid-state NMR

Objectifs visés du stage (5 lignes max) :
Nickel-binding motifs in proteins are usually made up of multiple histidine and/or cysteine residues. CooT is unusual as the binding site is formed by the dimerization of the protein that only contains a single strictly conserved cysteine. The goal of this internship is to use Dynamic Nuclear Polarization (DNP)-enhanced solid-state NMR to investigate precisely this original metal-binding site. The sensitivity enhancement brought by DNP should compensate for the low concentration required for the homodimer formation.

Intérêts pédagogiques et compétences visées (5 lignes max) :
The originality of the project relies on the use of DNP, an innovative NMR technique whose sensitivity and selectivity for binding sites should be best suited for the study of nickel binding to CooT. The approach will be strongly multidisciplinary and conducted in close collaboration with the Laboratory of Chemistry and Biology of Metals (LCBM), specialized in biochemical and structural characterization of metalloproteins. Targeted competences are in biochemistry, structural biology and NMR.

Résumé : Metalloenzymes often contain multi-metallic active sites whose biogenesis requires specific maturation pathways. Thus, the active site of the carbon monoxide dehydrogenase (CODH), a key enzyme of carbon metabolism that reversibly catalyzed the reduction of CO_2 in CO, is composed of a $NiFe_4S_4$ center, unique in biology. Its still unknown detailed biosynthesis pathway requires three poorly characterized nickel proteins. One of them, CooT is a small protein of 66 residues, which contains a single cysteine (Cys2) essential for Ni-binding. Although a crystal structure has been obtained by LCBM, the precise nature of the ligands as well as the metal coordination mode in the protein are still unknown. The high flexibility of the N-terminal region as well as the protein propensity to form biologically irrelevant tetramers in concentrated solutions makes the structural study of CooT by standard NMR and X-ray diffraction difficult. The use of solid-state NMR combined with DNP hyperpolarization should provide enough sensitivity and selectivity for the further precise investigation of CooT nickel binding site.

Approches & matériels utilisés (5 lignes max) :
^{13}C, ^{15}N isotopic labeled CooT will be produced following established overproduction protocols in *E. coli*. Standard and DNP-enhanced solid-state NMR experiments will be conducted on the two DNP spectrometers of the lab. The sensitivity enhancement brought by DNP is expected to be essential for the detection of the low concentrated CooT samples required for the dimer formation. A recently developed selective DNP method will be used to focus on the binding site.

Domaines de compétences souhaitées du candidat (3 lignes max):
This internship will be performed in strong collaboration between MEM and LCBM. Competences at the Chemistry-Biology interface are therefore required.