



	Experiment title: <b>Experiment title: NiSOD mimics based on peptide-like ligands. Correlating coordination and catalytic activity.</b>	<b>Experiment number:</b> 30-02 1143
<b>Beamline:</b> BM30	<b>Date of experiment:</b> from: 8/11/2018 to: 12/11/2018	<b>Date of report:</b> 01/02/2021
<b>Shifts:</b> 12	<b>Local contact(s):</b> Olivier Proux	<i>Received at ESRF:</i>
<b>Names and affiliations of applicants</b> (* indicates experimentalists):  <b>Pascale Delangle*</b> , SyMMES, UMR 5819 UGA CEA CNRS <b>Jérémy Domergue*</b> , SyMMES & DCM <b>Carole Duboc*</b> , DCM, UMR 5250, UGA CNRS <b>Jacques Pécaut*</b> , SyMMES, UMR 5819 UGA CEA CNRS		

## Report:

### Objective of the experiment:

This proposal aims at proving the coordination sphere of the Ni ion by XAS in complexes with peptide-based ligands that mimic the active site of the NiSOD enzyme.[1-3] The SOD catalyzes the dismutation of  $O_2^{\cdot-}$  into  $H_2O_2$  and  $O_2$  in order to protect cells against  $O_2^{\cdot-}$ . Different classes of SODs were identified. Importantly, the NiSOD is found in pathogenic bacteria but not in humans. Targeting the NiSOD is thus a promising approach to develop antibiotics but also potential antioxidant metallodrugs. The project of J. Domergue (PhD 2016-2019) aimed at developing active NiSOD mimics, based on peptide-like ligands to obtain efficient SOD-like catalysts and to contribute to the understanding of the catalytic mechanism of the NiSOD. He already obtained active complexes. Some of them were fully characterized by XAS and other spectroscopies and the results with a first tripodal sulfur ligand supported by XAS were published in Inorganic Chemistry in 2019.[4] This second series of XAS experiment has been designed to confirm and refine previous results from experiment 30-02 1132 performed in 2017 and to define structure/reactivity relationships with a larger series of compounds, derived from the ATCUN peptide motif.

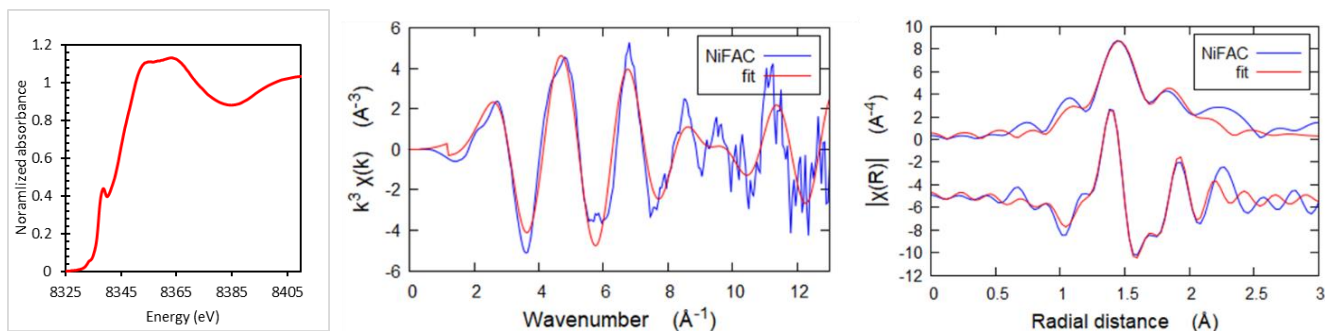
### Results:

Ni K-edge XAS spectra were acquired for 9 different samples of Ni(II) complexes with ATCUN-like peptides in water (HEPES 20 mM, NaCl 100 mM, pH 7.4)/glycerol 80/20 v/v mixtures at low temperature (10 K): **NiCAC**, (CAC = Cysteine-Alanine-Cysteine, **NiAcCAC**, Ni FAC (FAC = Phenylalanine-Alanine-Cysteine), **NiHAC** (HAC = Histidine-Alanine-Cysteine) and **NiCHC** (CHC = Cysteine-Histidine-Cysteine) and **NiCE(H)C** (Cysteine-Glutamate( $\gamma$ Histidine)-Cysteine). Some complexes were also acetonitrile to check whether the structure of the Ni(II) complex was solvent dependent.

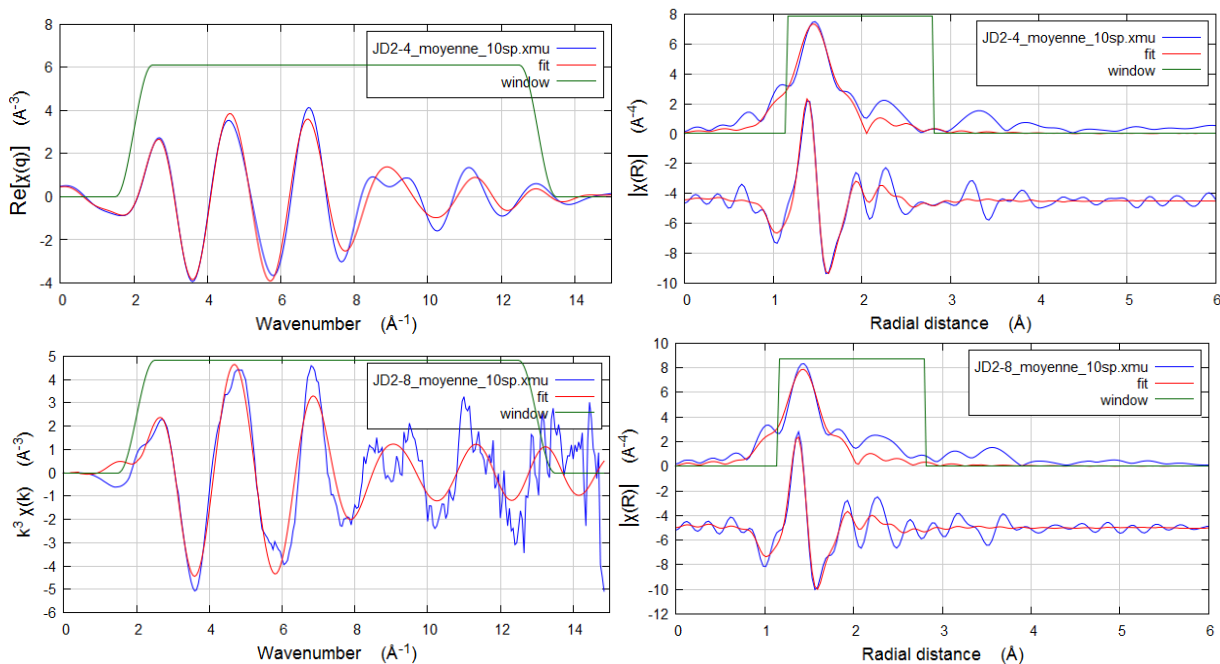
The XANES spectra evidence that all of our complexes have a **square planar geometry like in the enzyme**<sup>4</sup>, as both the  $1s \rightarrow 4p_z$  and  $1s \rightarrow 3d$  transitions can be seen in the pre-edge region (Fig. 1. Left).

The EXAFS data confirm this assignment. For example, the best fit obtained for NiFAC leads to three different shells: 1 S for the thiolate, 2 N for the amidates and 1 N for the amine. The calculated distances (Ni-S=2.18 Å, Ni-N=1.84 Å, Ni-NH<sub>2</sub>=1.92 Å) are close to those reported in the literature for such a coordination and close to the distances found in the enzyme NiSOD itself (Fig. 1).

A second interesting point consisted in studying the potential axial coordination of His and the comparison of the spectra in aqueous or organic solution (Fig. 2). Indeed the structure of the enzyme shows that a histidine is situated on axial direction but not coordinated to Ni(II). The results on NiHAC demonstrate that the square planar coordination is conserved with this ligand containing an imidazole potential ligand with no axial coordination. Moreover, EXAFS spectra recorded in water and acetonitrile are clearly superimposable demonstrating no structural difference.



**Fig. 1.** NiFAC data (left) XANES spectrum; (middle)  $k^3$  EXAFS spectrum, (right) modulus and imaginary parts of the Fourier transformed  $k^3$  spectrum (blue) and the simulations (red). Simulation parameters:  $\Delta E_0 = 6(2)$ , shell no. 1: Ni-S,  $n = 1$ ,  $r = 2.18(1) \text{ \AA}$ ,  $\sigma^2 = 0.0014(7) \text{ \AA}^2$ ; shell no. 2: Ni-N,  $n = 3$ ,  $r = 1.867(8) \text{ \AA}$ ,  $\sigma^2 = 0.0022(6) \text{ \AA}^2$ ;  $R = 1.6\%$ ;  $\chi^2_{\nu} = 9$ .



**Fig. 2.** (left)  $k^3$  EXAFS spectrum in water (up) and AcN (bottom), (right) modulus and imaginary parts of the Fourier transformed  $k^3$  spectrum (blue) and the simulations (red) in water (up) and in AcN (bottom). Simulation parameters in water :  $\Delta E_0 = 1.61$ ,  $s_0^2 = 0.87$ , shell no. 1: Ni-S,  $n = 1$ ,  $r = 2.16 \text{ \AA}$ ,  $\sigma^2 = 0.00657 \text{ \AA}^2$ ; shell no. 2: Ni-N,  $n = 3$ ,  $r = 1.87 \text{ \AA}$ ,  $\sigma^2 = 0.00482 \text{ \AA}^2$ ; shell no. 3: Ni-C,  $n = 6$ ,  $r = 2.77 \text{ \AA}$ ,  $\sigma^2 = 0.05683 \text{ \AA}^2$ ,  $R = 2.5\%$ . Simulation parameters in AcN:  $\Delta E_0 = 1.64$ ,  $s_0^2 = 0.87$ , shell no. 1: Ni-S,  $n = 1$ ,  $r = 2.15 \text{ \AA}$ ,  $\sigma^2 = 0.00693 \text{ \AA}^2$ ; shell no. 2: Ni-N,  $n = 3$ ,  $r = 1.84 \text{ \AA}$ ,  $\sigma^2 = 0.00418 \text{ \AA}^2$ ; shell no. 3: Ni-C,  $n = 6$ ,  $r = 2.72 \text{ \AA}$ ,  $\sigma^2 = 0.05801 \text{ \AA}^2$ ,  $R = 4.5\%$ .

## Conclusion:

Thanks to these experiments, we obtained crucial information on the Ni(II) environment in the series of active SOD complexes developed in our project. All the complexes demonstrate a square planar geometry around the metal with Ni-S and Ni-N distances close to the enzyme's active site. EXAFS data evidence no coordination of the histidine in axial position similarly to the NiSOD, and no influence of the solution, water or organic medium. A first article has been published for the tripodal trisulfur ligand and the data obtained in this experiment are currently being written for publication.

## References:

- [1] *J. Biol. Chem.*, **1997**, 272, 18515-18517
- [2] *Annu. Rev. Biochem.*, **1975**, 44, 147-159
- [3] *Curr. Opin. Chem. Biol.*, **2004**, 8, 162-168
- [4] *Mononuclear Ni(II) complexes with a S3O coordination sphere based on a tripodal cysteine-rich ligand: pH tuning of the SOD activity*, Domergue, J.; Pécaut, J.; Proux, O.; Lebrun, C.; Gateau, C.; Le Goff, A.; Maldivi, P.; Duboc, C.; Delangle, P., *Inorg. Chem.* **2019**, 58, 12775-12785.
- [5] *A bio-inspired Ni<sup>II</sup> complex with an ATCUN-like motif as a catalyst for superoxide dismutase activity in water and identification of active intermediate species*. Domergue, J.; Guinard, P.; Douillard, M.; Pécaut, J.; Proux, O.; Lebrun, C.; Delangle, P.; Le Goff, A.; Maldivi, P.; Duboc, C. *Submitted soon*.