

**Experiment title:**

Co local structure in CnrX, a metal-sensor triggering the resistance to cobalt and nickel in the environmental strain *Cupriavidus metallidurans* CH34.

Experiment number:

30-02-973

Beamline:

BM30B

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Received at ESRF:

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Report:

CnrX is the metal-sensor and the signal modulator of the three-protein transmembrane signal transduction complex CnrYXH of *Cupriavidus metallidurans* CH34 involved in the set up of cobalt and nickel resistance. We have determined the atomic structure of the soluble domain of CnrX in its Ni-, Co- or Zn-bound form [1]. Ni and Co ions elicit a biological response while the Zn-bound form is inactive. The structures reveal the topology of the intra- and inter-protomer interactions and the ability of the metal-binding sites to fine-tune the packing of CnrX dimer as a function of the bound metal.

In solution, CnrXs is titrated by 4 Co-equivalent and exhibits an unexpected intense band at 384 nm that was detected neither by single-crystal spectroscopy nor under anaerobiosis. A detailed spectroscopic study including X-ray absorption spectroscopy was performed to further characterize the different metal-binding sites of CnrXs and those of NccX, a full-length membrane bound protein, analogue of CnrX.

The data from a combination of spectroscopic techniques (spectrophotometry, electron paramagnetic resonance, X-ray absorption spectroscopy) showed that two sites correspond to those identified by crystallography. The two extra binding sites accommodate Co(II) in an octahedral geometry in the absence of oxygen and are occupied in air by a mixture of low-spin Co(II) as well as EPR-silent Co(III). These extra sites, located at the N-terminus of the protein, are believed to participate to the formation of peroxo-bridged dimers. Accordingly, we hypothesize that the intense band at 384 nm relies on the formation of a binuclear m-peroxo Co(III) complex. These metal binding sites are not physiologically relevant since they are not detected in full-length NccX, the closest homologue of CnrX. X-ray absorption spectroscopy demonstrates that NccX stabilizes Co(II) in two-binding sites similar to those characterized by crystallography in its soluble counterpart. Nevertheless, the original spectroscopic properties of the extra Co-binding sites are of interest because they are susceptible to be detected in other Co-bound proteins.

These results have been published under the following reference:

Trepreau J., de Rosny E., Duboc C., Sarret G., Petit-Hartlein I., Maillard AP., Imberty A., Proux O. and Covès J. (2011)

Biochemistry **50**, 9036-9045.

Spectroscopic characterization of the metal-binding sites in the periplasmic metal-sensor domain of CnrX from *Cupriavidus metallidurans* CH34.

Here are the tables and figures related to the X-ray absorption spectroscopy experiments:

Table 1: Structural environment for Co in 2-Co-bound forms of H32A-CnrXs, NccX and E63Q-CnrXs obtained from EXAFS data analysis.

	Atom	N	R (Å)	σ^2 (Å ²)	ΔE (eV) ¹	R factor
H32-CnrXs 2 Co	His ²	3.0±0.2	2.11±0.04	0.004	8.0	1.6 · 10 ⁻²
	O	2.0±0.2	2.13±0.07	0.009	8.0	
	S	0.9±0.1	2.57±0.02	0.005	8.0	
NccX 2 Co	His ²	3.0±0.2	2.09±0.02	0.005	8.2	2.1 · 10 ⁻²
	O	2.0±0.2	2.13±0.04	0.003	8.2	
	S	1.1±0.1	2.57±0.02	0.010	8.2	
E63Q-CnrXs 2 Co	His ² (Co(III)) ³	0.5±0.2	1.93±0.003	0.003	2.0	5.0 · 10 ⁻³
	N/O (Co(III)) ³	2.0±0.2	1.93±0.003	0.003	2.0	
	His ² (Co(II))	2.0±0.1	2.13±0.005	0.003	10.0	

N : Number of atoms, R : interatomic distance, s^2 : Debye-Waller factor, ΔE (eV): difference between the theoretical and experimentally determined threshold energies, R factor: residual between fit and experiment.¹ Values of 9 ± 1 eV correspond to Co(II), and values of 2 ± 1 eV correspond to Co(III).² Including multiple scattering within the imidazole ring.³ A single Co(III)-N was used for the fit, which was then separated between Co(III)-His ($N = 0.5$ based on the multiple scattering contributions) and Co(III)-N/O

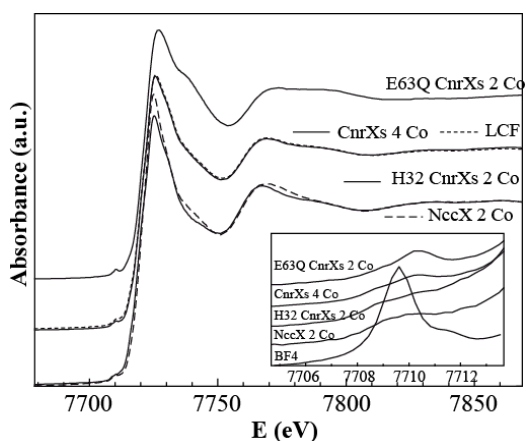


Figure 5: Co K -edge XANES spectra for 2-Co-bound E63Q-CnrXs, 4-Co-bound CnrXs, 2-Co-bound H32A-CnrXs and NccX. The dotted line superimposed on 4-Co-bound CnrXs is a linear combination fit obtained with 62% 2-Co-bound H32A-CnrXs + 36% 2-Co-bound E63Q-CnrXs. Insert: close-up view the pre-edge feature and comparison with Co(2-methylimidazole)₄(BF₄)₂.

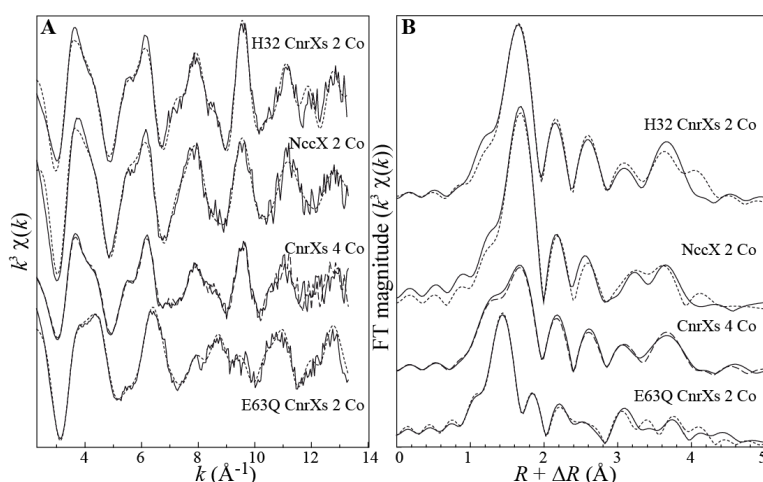


Figure 6: Co K -edge EXAFS spectra (A) and Fourier transforms moduli (B) for 2-Co-bound E63Q-CnrXs, 4-Co-bound CnrXs, 2-Co-bound H32A-CnrXs and 2-Co-bound NccX. The dotted lines superimposed on 2-Co-bound E63Q-CnrXs, 2-Co-bound H32A-CnrXs and 2-Co-bound NccX are multi-shell fits, and the dashed line superimposed on 4-Co-bound CnrXs is a linear combination fit obtained with 56% 2-Co-bound H32A-CnrXs + 32% 2-Co-bound E63Q-CnrXs.

[1] Trepreau, J., Girard, E., Maillard, A.P., de Rosny, E., Petit-Haertlein, I., Kahn, R., and Covès, J. (2011) *J. Mol. Biol.* **408**, 766-779.

Structural basis for metal sensing by CnrX,