



	Experiment title: Probing the Effect of LPMO "Gatekeeper" Residue Identity on Cu Site Stabilization via Cu K-edge X-ray Absorption Spectroscopy	Experiment number: CH-6333
Beamline: ID26	Date of experiment: from: 08 Mar 2022 to: 15 Mar 2022	Date of report: 01 Sept 2022
Shifts: 12	Local contact(s): Viktoriia Saveleva	<i>Received at ESRF:</i>
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Report:

Lytic polysaccharide monooxygenases (LPMOs) are a class of C–H activating Cu metalloenzymes which play an important role in biomass decomposition processes needed to maintain a net-neutral carbon cycle. Recent computational investigations into the reaction mechanism of LPMO identify a crucial second sphere residue, termed the “gatekeeper” residue, as playing a crucial role by engaging the co-substrate in strained conformations to facilitate activation of the O–O bond. In our research, we had recently prepared four variants of *NcAA9C* LPMO which focused on mutations of the gatekeeper residue. For each variant, both the Cu(I) and Cu(II) congeners were prepared. Data collection on two of the variants (WT and Q164E) in PFY-detected XAS configuration suggested differences Cu(I) congeners of the two species, while the Cu(II) data were similar. However, data collection on other lower-concentration samples were hampered by the presence of background metallic Cu signal (presumed from the cryostat) and strong ice diffraction issues. In order to circumvent these issues, we chose to collect at ID26 using a HERFD configuration.

The ID26 beamline was configured for a HERFD experiment and equipped with Si(311) double crystal monochromator for upstream energy selection. The detector comprised of a Johann arrangement of five Si(111) analyzer crystals and an Avalanche Photodiode windowed to the Cu $K\alpha$ emission region. Samples were housed in a liquid He cryostat held at 30 K during data collection. The choice of the Si(311) monochromator crystals was made for two reasons: (1) the increased energy resolution relative to the Si(111) would help identify subtle shifts in the XANES spectra between the mutants and (2) the decrease in flux can be somewhat beneficial for highly photosensitive Cu(II) samples, which easily photoreduce to Cu(I) under X-ray flux. Our research group has previously utilized such a setup to great success with data taken on the Fe-containing sMMO metalloenzyme (data collected prior to the storage ring upgrade at ESRF).¹ However, it was found that using the same setup for Cu HERFD-XAS (following the storage ring upgrade) resulted in beam instability during scans as the monochromator sweep progressed through energy of the incident beam. This consequently led to EXAFS-region data appearing as uncharacteristically “wobbly” (Figure 1) which would not be possible to fit.

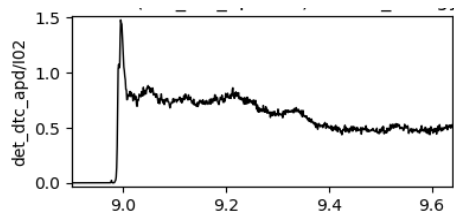


Figure 1. Representative EXAFS scan depicting consequences of beam instability. The EXAFS region exhibits significant modulation of the data, making fitting by standard methods untenable.

It was nonetheless possible to collect XANES data of superior resolution (relative to previously collected data using a PFY-detected configuration). The XANES region of the Cu(I) congeners for all four variants were collected, and the improved energy resolution revealed a subtle shift to higher energy for the $1s \rightarrow 4p$ feature of the Q164E mutant compared to the other three variants (WT, Q164N, Q164D) (Figure 2).

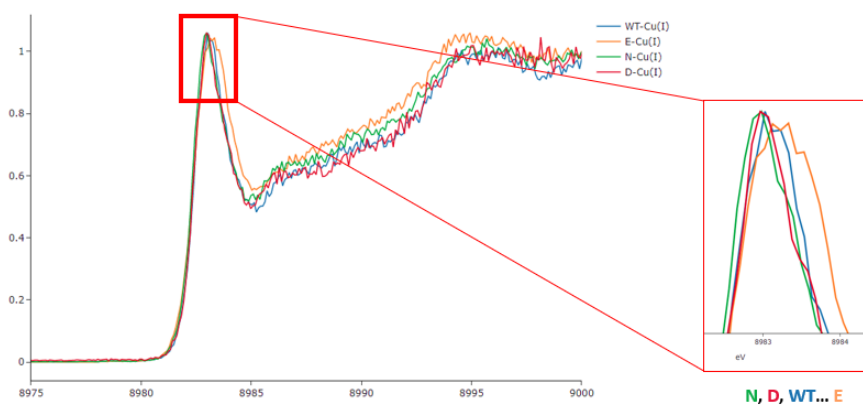


Figure 2. XANES region scans of the four variants in Cu(I) oxidation state: WT (blue), Q164N (green), Q164D (red), and Q164E (orange). The improved resolution reveals a subtle upward energy shift in the $1s \rightarrow 4p$ feature of the Q164E mutant.

In addition to the Cu-LPMO data collected, $K\alpha$ -detected HERFD-XAS data were collected on small molecule tripod-supported Co complexes of varying oxidation states with either a ligand-centered radical or close-shell complex. This data has been published.² Finally $K\beta$ -detected HERFD-XAS and NR emission data were collected on Fe-imido and Fe-nitride complexes of varying oxidation states (Figure 3)

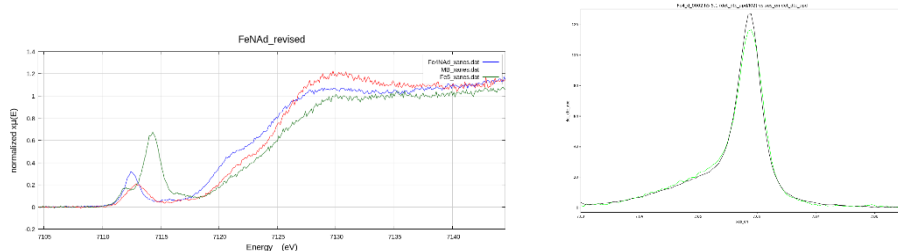


Figure 3. (left) Fe K-beta-HERFD of Fe(IV)-imido precursor (blue), unstable freeze-trapped Fe(V)-imido species (red) containing 30% of the precursor (as found by Mössbauer) and the Fe(V)-nitride decay product (green). (right) Non-resonant $K\beta$ mainline of an iron-nitride and an imido complex in (IV) oxidation state, revealing both to be low-spin, consistent with the observed Mössbauer data on the same samples.

References:

- (1) Cutsail, G. E.; Banerjee, R.; Zhou, A.; Que, L.; Lipscomb, J. D.; DeBeer, S. High-Resolution Extended X-Ray Absorption Fine Structure Analysis Provides Evidence for a Longer $\text{Fe} \cdots \text{Fe}$ Distance in the Q Intermediate of Methane Monooxygenase. *J. Am. Chem. Soc.* **2018**, *140* (48), 16807–16820. <https://doi.org/10.1021/jacs.8b10313>.
- (2) Mao, W.; Fehn, D.; Heinemann, F. W.; Scheurer, A.; van Gestel, M.; Jannuzzi, S. A. V.; DeBeer, S.; Munz, D.; Meyer, K. Umpolung in a Pair of Cobalt(III) Terminal Imido/Imidyl Complexes. *Angew. Chemie Int. Ed.* **2022**, *61* (36). <https://doi.org/10.1002/anie.202206848>.