Characterisation of LPMO Intermediates using XAS and Rapid Freeze Quenching

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Lytic Polysaccharide Monooxygenases (LPMOs) are a family of naturally occurring copper-containing enzymes which have the ability to be able to perform the oxidative saccharification of cellulose. Cellulose is the most abundant natural polymer on Earth and is therefore an ideal potential feedstock for the production of second-generation biofuels [1]. Understanding of the LPMO catalytic mechanism is fundamental in developing biofuels as an attractive source of renewable energy.

Stopped-flow spectrophotometry has shown that the catalytic mechanism of LPMOs includes the formation of short-lived intermediate species when reacted with an oxidant. The development of anaerobic rapid freeze quenching techniques for the preparation of XAS samples has led to the characterisation by HERFD-XANES of a copper histidyl-radical species which forms and decays on a millisecond timescale. On the decay of the histidyl-radical intermediate, a more stable tyrosyl intermediate is formed (Figure 1). It is suggested that the formation and decay of these intermediates is part of a repair mechanism which mitigates oxidative damage in tandem with the elusive catalytic reaction mechanism of LPMOs, allowing for more efficient turnover [2]. This mechanism could be significant in a number of metal-histidine oxygenase complexes.

![Figure 1: Reaction pathways of Cu(I)-LsAA9 reacting with peroxides in the absence of substrate [2].](image)

The preparation of freeze quenched LPMO samples and XAS data collection has many challenges including the rate of photoreduction of copper(II) species, the anaerobic conditions and fast rate of decay of LPMO radical intermediates. The insights we can gain from the combination of anaerobic rapid freeze quenching and XAS in the study of LPMOs have profound applications in the production of biofuels as well as in oxygenase and copper chemistry.

2) J. Zhao et al., Research Square, 2022. [Preprint]. DOI: 10.21203/rs.3.rs-1350705/v1