MS06-2-5 Exploring the structure and mechanism of heme peroxidases using SFX and multicrystal composite approaches
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M. Rozman [1], D. Svistunenko [1], M. Wilson [1], D. Axford [2], A. Ebrahim [2], T. Tosha [3], H. Sugimoto [3], I. Tews [4], R. Owen [2], J. Worrall [1], M. Hough [2]
[1]University of Essex - Colchester (United Kingdom), [2]Diamond Light Source - Didcot (United Kingdom), [3]RIKEN SPring8 Center - Harima (Japan), [4]University of Southampton - Southampton (United Kingdom)

Abstract
Heme peroxidases access Fe(III) and Fe(IV) states of Fe in their catalytic mechanism. These states are exquisitely sensitive to radiation damage, creating a significant challenge to obtain intact structures and hence to relate these accurately to mechanistically relevant states of the enzymes. Dye decolourising peroxidases have a largely unknown physiological function although certain enzymes of this class have been suggested to have significant potential industrial application in the breakdown of recalcitrant polysaccharides such as lignin as part of the production of biofuels.
We used serial femtosecond crystallography at SACLA with fixed targets and extruder sample delivery to obtain intact room temperature structures of the Fe(III) and Fe(IV) states of several different dye-decolourising peroxidases from Streptomyces lividans. Structures were corroborated using multicrystal datasets at 100 K for each state, where the UV-visible spectrum of each crystal was measured prior to and following X-ray data collection to validate the redox state in the resulting multicrystal dataset and structure. Moreover, we explored the effects of X-ray dose on the crystals, observing elongation of Fe(III)-OH2 and Fe(IV)=O bond lengths as a function of the absorbed dose.
The results of these studies [1,2], in combination with extensive kinetic and spectroscopic data reveal key features of enzyme mechanisms and differences between the three Streptomyces lividans Dyps studied including the role of 'dry' versus 'wet' distal heme pockets and the manner in which mechanism is tuned to utilise aspartate or arginine residues for the rates enhancement of peroxide heterolysis.

References
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Aspartate or arginine? Validated redox state X-ray structures elucidte mechanistic subtleties of FeIV = O formation in bacterial dye-decolorizing peroxidases (2021) Marina Lučić, Michael T. Wilson, Dimitri A. Save file Svistunenko, Robin L. Owen, Michael A. Hough & Jonathan A. R. Worrall. JBIC Journal of Biological Inorganic Chemistry 26, 743–761