Toward geometric structures of oxidized cofactors and high-valent metal-oxygen intermediates in di-metal proteins by femtosecond XFEL crystallography

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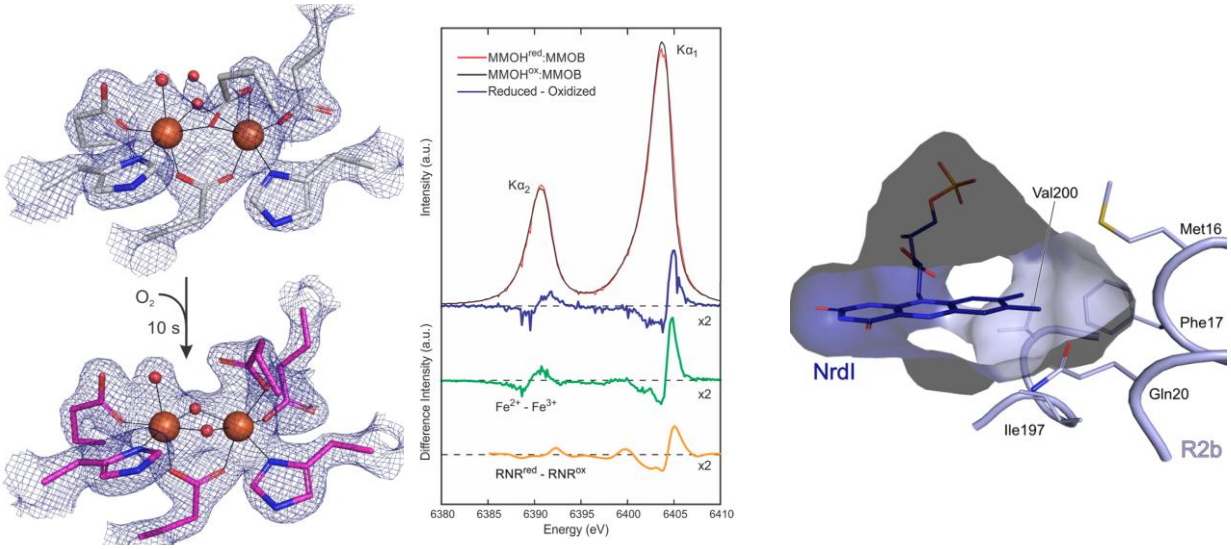
The archetypical di-metal carboxylate proteins bind two ferrous (Fe(II)) ions to produce an oxygen-activating cofactor used for some of the most chemically challenging oxidations observed in nature. The two most studies examples are soluble methane monooxygenase and the aerobic ribonucleotide reductase R2. Proteins from the same superfamily utilizing Mn/Mn or Mn/Fe cofactors have also been discovered[1]. In some cases, additional cofactors such as flavins initially reduce molecular oxygen to superoxide that in turn oxidizes the metal site. Upon reaction with molecular oxygen or superoxide, several high-valent metal-oxygen intermediates are generated.

These intermediates are highly interesting for both basic and applied science but obtaining their global geometric structures has proven very challenging.

In close collaboration with scientists at the LCLS, LBNL and the university of Minnesota, a conveyor-belt sample injector-based experimental setup has been developed that allows micrometer-sized crystals to be incubated with oxygen for a defined period of time before exposure to the free-electron laser X-ray beam[2]. This setup allows varying the time for intermediate trapping while the use of femtosecond XFEL crystallography eliminates the effect of X-ray photoreduction on obtained data. Simultaneous XES also allows *in situ* oxidation state determination of probed intermediates.

This setup and its use to obtain high-resolution global geometric structures of high-valent intermediates will be discussed, as well as our recent progress defining radiation undamaged structures of soluble methane monooxygenase[3] and ribonucleotide reductase proteins[4,5].



[1] Högbom M. *Metallomics*, **3**,110-20 (2011)
[2] F.D. Fuller et al. *Nature Methods*, 14(4):443-449 (2017)
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