Ferritin-like dinuclear metalloenzymes studied by XFEL and MicroED

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Ferritin-like proteins share a common fold coordinating a pair of metal ions, and perform a diverse set of reactions. In our laboratory, we study several members of this superfamily, including the soluble methane monooxygenase (sMMO), the ribonucleotide reductase (RNR) and the R2-like ligand-binding oxidase (R2lox), which all rely on dinuclear iron and/or manganese cofactors to catalyse challenging oxygen-dependent reactions. For example in RNR class Ic, a Mn(II)Fe(II) heterodinuclear centre initially provides all four electrons required for complete O_2 reduction. It results in a Mn(IV)Fe(IV) intermediate which undergoes a one-electron reduction to form the Mn(IV)Fe(III) active state of the protein. Although still debated, the inorganic cores of this RNR after O_2 exposure are to date believed to be Mn(IV)(μ-O)$_2$Fe(IV) and Mn(IV)(μ-O)(μ-OH)Fe(III).

In order to study these metalloenzymes in action, we aim to capture snapshots of their metal cofactors in different redox states by serial femtosecond X-ray crystallography and simultaneous X-ray emission spectroscopy (XES) at X-ray free-electron laser (XFEL) sources [1]. Using a drop-on-tape sample delivery system with *in situ* O_2-incubation at room temperature, we obtained high-resolution XFEL crystal structures coupled with XES data [2]. In parallel, we use microcrystal electron diffraction (MicroED), an emerging method of cryo-electron microscopy (cryo-EM), in an attempt to better characterise enzyme metal centres [3]. Here, I will present how we prepare the microcrystal samples required in these two approaches, and discuss our recent structural results on sMMO, RNR and R2lox in the light of the current models proposed by spectroscopy and single-crystal X-ray crystallography. Finally, I will talk about our discovery of a metal-independent RNR and how it raises questions about the use of metals in some microorganisms [4].

[1] Fuller FD, Gul S, Chatterjee R, Burgie ES, Young ID, Lebrette H *et al.* Drop-on-demand sample delivery for studying biocatalysts in action at X-ray free-electron lasers. *Nat Methods* 2017, 14(4):443-449.
[2] Srinivas V, Banerjee R, Lebrette H *et al.* High resolution XFEL structure of the soluble methane monooxygenase hydroxylase complex with its regulatory component at ambient temperature in two oxidation states. *J Am Chem Soc* 2020, 142(33):14249-14266.
[3] Xu H*, Lebrette H* *et al.* Solving a new R2lox protein structure by micro-crystal electron diffraction. *Sci Adv* 2019, 5(8):eaax4621.
[4] Srinivas V*, Lebrette H* *et al.* Metal-free ribonucleotide reduction powered by a DOPA radical in Mycoplasma pathogens. *Nature* 2018, 563(7731):416-420.