

X-ray crystallography and spectroscopy of metalloenzymes using drop-on-tape method

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The development of XFELs has opened up opportunities for studying the dynamics of biological systems beyond what is possible at synchrotron radiation (SR) sources. Intense XFEL pulses enable us to apply both X-ray diffraction and X-ray spectroscopic techniques to dilute systems or small protein crystals. By taking advantage of ultra-bright femtosecond X-ray pulses, one can also collect the data under functional conditions of temperature and pressure, in a time-resolved manner, after initiating reactions, and follow the chemical dynamics during catalytic reactions and electron transfer. Such an approach is particularly beneficial for biological materials and aqueous solution samples that are susceptible to X-ray radiation damage. We have developed spectroscopy and diffraction techniques necessary to fully utilize the capability of the XFEL X-rays for a wide-variety of metalloenzymes, and to study their chemistry under functional conditions. One of such methods is simultaneous data collection for X-ray crystallography and X-ray spectroscopy, to look at the overall structural changes of proteins and the chemical changes at metal catalytic sites. The other method is soft X-ray absorption spectroscopy of metalloenzymes by developing a spectrometer that can discriminate between the metal signal and the large background signal, making possible the study of dilute biological systems under ambient conditions. In parallel to the detection techniques, we have also developed an efficient sample delivery method that involves deposition of droplets on a conveyor belt [1]. This 'Droplet on Tape' (DOT) method, delivers a single drop of the crystal suspension or solution sample onto a tape, which then can be transported to the X-ray intersection point with a variable delay in time. In the process, the sample is photochemically or chemically activated at various time-delays to capture reaction intermediates with crystallography and spectroscopy. The setup is suitable for both diffraction and spectroscopy data collection, simultaneously.

We have used the above techniques to study (i) photochemical activation of the water oxidation reaction of the Photosystem II multi-subunit protein complex, in which the Mn₄CaO₅ cluster catalyzes the reaction [2], (ii) the oxygen activation of ribonucleotide reductase (RNR), and (iii) other metalloenzymes. The current status of this research and the mechanistic understanding of these metalloenzymes based on the X-ray techniques is presented.

[1] Fuller F. D. et al. (2017) Nature Methods, doi:10.1038/nmeth.4195.

[2] Young I. D. et al. (2016) Nature 540, 453-457.

Keywords: [X-ray spectroscopy](#), [metalloenzymes](#), [oxygen activation](#)