Using femtosecond crystallography to look at a radical trying to break free

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Radicals get bad press as they are considered responsible for damaging cells, causing aging and diseases. However, radicals are also essential to many enzymes involved in fundamental biological processes. Half a century ago, the tyrosyl radical in the ribonucleotide reductase (RNR) was the first stable protein radical to be observed. RNR is a drug target for both cancer and infectious diseases as it provides the only pathway for de novo synthesis of deoxyribonucleotides, the building blocks of DNA. Aerobic RNR relies on the R1 subunit to perform the ribonucleotide reduction and on the ferritin-like R2 subunit to produce a catalytic radical upon oxygen activation. The radical is translocated between the subunits via reversible proton-coupled electron transfer.

We are interested in several subclasses of aerobic RNRs with different means of radical generation, which depend on dinuclear iron and/or manganese cofactors (except in particular case of metal-independent RNR such as in mycoplasmas [1]). In order to study these metalloenzymes in action, we aim to capture snapshots of their cofactors in different redox states by serial femtosecond X-ray crystallography and simultaneous X-ray emission spectroscopy at X-ray free-electron laser (XFEL) sources [2]. Using a drop-on-tape sample delivery system with in situ O_2-incubation at room temperature, we could obtain high-resolution XFEL crystal structures [3,4], including the structure of a R2 subunit with an intact radical present in its core [5]. Here, I will present our recent results and discuss the advantages of XFEL to study metalloenzymes.

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[2] Fuller FD *et al*. *Nat Methods* **2017**, 14(4):443-449.
[3] Srinivas V *et al*. *J Am Chem Soc* **2020**, 142(33):14249-14266.
[4] John J *et al. Elife* **2022**, 11:e79226.
[5] Lebrette H*, Srinivas V* *et al. Science* **2023**, 382(6666):109-113.