

Damage-free structures of green copper nitrite reductase obtained by neutron crystallography and XFEL

S. Antonyuk

Molecular Biophysics Group, ISMIB, Faculty of Health and Life Sciences; University of Liverpool, UK

S.Antonyuk@liverpool.ac.uk

Copper-containing nitrite reductases (CuNiRs) that convert NO_2 to NO are of central importance in nitrogen-based energy metabolism [1]. These metalloenzymes, like all redox enzymes, are very susceptible to radiation damage from the intense synchrotron radiation by X-rays, that are used to obtain structures at high resolution. Understanding the chemistry that underpins the enzyme mechanisms in these systems usually requires atomic resolutions of better than 1.2 Å. The damage-free structure of the resting state of one of the most studied CuNiRs was obtained by X-ray free-electron laser (XFEL) and neutron crystallography, which allows direct comparison of neutron, XFEL structural data [2] and atomic resolution X-ray structural data used to obtain the most accurate (atomic resolution with unrestrained SHELX refinement) structure.

It was demonstrated that Asp_{CAT} (Asp₉₈) and His_{CAT} (His₂₅₅) are deprotonated in the resting state of CuNiRs at pH values close to the optimum for activity (Fig.1).

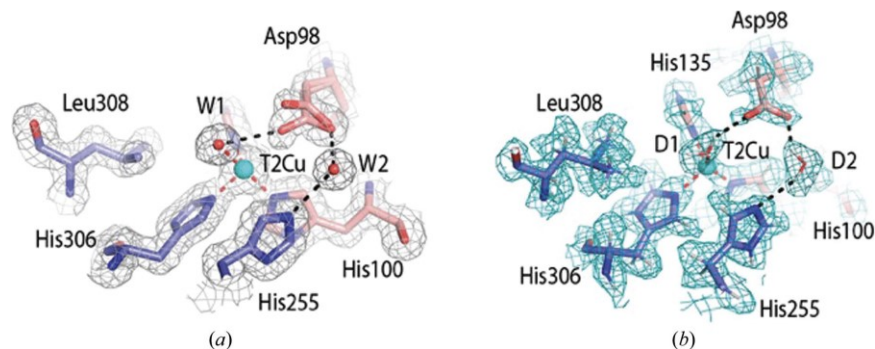


Figure 1. The T2Cu site of AcNiR determined by SF-ROX (a) and neutron crystallography (b).

[1] Zumft, W. G. (1997). *Microbiol. Mol. Biol. Rev.* **61**, 533

[2] Halsted, T.P., Yamashita, K., Gopalasingam, C. C., Shenoy, R.T, Hirata, K., Ago, H., Ueno, G., Blakeley, M.P., Eady, R R.; Antonyuk, S.V., Yamamoto, M., Hasnain, S. S. (2019). *IUCrJ* **6**, 761

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