A017b-06-240823

Spectroscopically validated multiple structures from one crystal (MSOX) and Damage Free atomic structures using XFEL for copper Nitrite Reductases

Samuel L. Rose[1], Svetlana V. Antonyuk[1], Robert R. Eady[1], Felix Ferroni[2], Takehiko Tosha[3], Masaki Yamamoto[3], Samuel Horrell[4], Robin Owen[4] and S. Samar Hasnain[1*]

Molecular Biophysics Group, Life Sciences Building, Institute of Systems, Molecular and Integrative Biology, Faculty of Health and Life Sciences, University of Liverpool, L69 7ZB, UK; [2] CONICET-CCT SANTA FE, Departamento de Física-FBCB-UNL, Ciudad Universitaria. Paraje El Pozo. Ruta Nac. 168 Km472, Argentina; [3]RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo, Hyogo, 679-5148, Japan; [4]Diamond Light Source, Harwell Science & Innovation Campus, Didcot, Oxfordshire, OX11 0DE

Many enzymes utilize redox-coupled centres for performing catalysis where the centres are used to control and regulate the transfer of electrons required for catalysis, whose untimely delivery can lead to a state incapable of binding the substrate i.e. a dead-end enzyme. Copper nitrite reductases (CuNiRs), which catalyse the reduction of nitrite to nitric oxide (NO), have proved to be a good model system for studying these complex processes including proton-coupled electron transfer and their orchestration for substrate binding/utilisation [1].

X-rays used to collect crystallographic data can in itself result in changes in the redox states of transition metals utilised by many biological systems including metalloproteins. This disadvantage has been harnessed to drive a complex chemical reaction requiring the delivery of an electron to the active site and recording the structural changes accompanying catalysis providing a 'real-time' structural movie of an enzymatic chemical reaction, a dream of enzymologist for decades. By coupling MSOX technique with single-crystal and solution optical spectroscopy, we show that the electron transfer between the T1Cu and T2Cu redox centres is heavily gated and show a structural movie capturing the bond-breakage, product formation and its release from the catalytic centre for CuNiRs from two organism [2].

Structures **f**ree from **r**adiation-**i**nduced **c**hemistry (FRIC structures) for proteins containing redox centres have become possible using single shot femtosecond pulses from X-ray Free Electron Lasers [3]. We have used high energy X-rays from SACLA to obtain atomic/sub-atomic resolution structures of three different nitrite reductases in a variety of functional states including substrate and product bound species alongside single crystal optical spectra. These spectroscopically validated very high resolution FRIC structures with unrestrained SHELXL refinement are providing unprecedented level of details including protonation states of key residues in the catalytic pocket. These new results will be reported at the Congress.

These recent developments would be reviewed with wider applicability in mind.

1 *New horizons in structure-function studies of copper nitrite reductase (2022) R. R. Eady & S. S. Hasnain,* ***Coordination Chemistry Reviews,*** *460. doi:10.1016/j.ccr.2022.214463*

2 *An unprecedented insight into the catalytic mechanism of copper nitrite reductase from atomic-resolution and damage-free structures (2021) S. L. Rose, S.V. Antonyuk, D. Sasaki, K. Yamashita, K. Hirata, Go Ueno, H. Ago, R. R. Eady, T. Tosha, M. Yamamoto, S. S. Hasnain* ***Science Adv.*** *7 eabd8523*

3 Single crystal spectroscopy and multiple structures from one crystal (MSOX) define catalysis in copper nitrite reductases. (2022*) S. L. Rose, S. Baba, H. Okumura, S. V. Antonyuk, D. Sasaki, T. M. Hedison, M. Shanmugam, D. J. Heyes, N. S. Scrutton, T. Kumasaka, T. Tosha, R. R. Eady, M. Yamamoto & S. S. Hasnain* ***Proc. of the National Academy of Sciences, 119****(30):e2205664119.*