Time-resolved studies of nitric oxide turnover in cytochrome *c* oxidase

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Cytochrome *c* oxidase (C*c*O) is a key enzyme in oxidative phosphorylation, where it couples the reduction of oxygen to water with proton pumping. Some C*c*Os can also reduce nitric oxide (NO) to nitrous oxide (N_2O). We study this reaction in the ba_3-type C*c*O from *Thermus thermophilus* using photolabile and pH-dependent NO donors to deliver NO directly into the active site. We follow changes after NO-release by serial crystallography and UV-Vis spectroscopy. I will present the first crystallographic models of NO bound ba_3-type C*c*O and discuss the reaction intermediates. In accordance with previous studies, we observe that NO turnover proceeds far slower than oxygen turnover [1], presenting both advantages and challenges for time-resolved crystallography. The work highlights current technical limitations when working under anaerobic conditions and triggering reactions with photocages for experiments at synchrotron and XFEL facilities.

[1] Giuffrè, A., Stubauer, G., Sarti, P., Brunori, M., Zumft, W.G., Buse, G., Soulimane, T., 1999. The heme-copper oxidases of Thermus thermophilus catalyze the reduction of nitric oxide: Evolutionary implications. Proc. Natl. Acad. Sci. U.S.A. 96, 14718–14723. https://doi.org/10.1073/pnas.96.26.14718