X-ray induced reduction of heme metal centers is protein-independent – implications for structural studies of redox sensitive proteins

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X-ray crystallography is one of the main resources to obtain information on the coordination of molecules within the active site of metalloproteins on an atomistic level. Based on ligand coordination, interatomic distances and relative positioning of catalytic amino acids enzymologists try to understand the underlying electronic reaction mechanism. Therefore the exact redox status and conformation of the cofactor in question is of utmost importance. Unfortunately the redox active nature of metal cofactors makes them especially susceptible to irradiation induced photoreduction, making structural information obtained by photo-reducing X-ray sources the least trustworthy [1,2].

Here we present a study of the pre-steady state reduction kinetics of X-ray induced photo-reduction of six different model heme proteins to identify a reasonable dose-limit for the collection of non-reduced datasets for redox-active metallo enzymes. Using online-UV-vis spectroscopy we examined the reduction kinetics of the heme cofactor to understand the impact of sample-derived variables (protein, crystallization conditions, crystal morphology and cofactor) and irradiation-derived variables (dose and dose rate). We can show that the reduction kinetics solely depend on the dose, irrespective of the sample-derived variables (Figure 1 A+B) and define a protein-independent dose-limit of 25 kGy, which corresponds to a 50% reduction. Furthermore we present a method of data collection and processing that allows collection of time-resolved low dose structures using standard macromolecular crystallography tools. Finally we present structures of a model heme protein (KpDyP) in different defined redox states. These structures show photoreduction induced rearrangements in water coordination and conformation of the catalytically relevant residue Asp 143 [3] (Figure 1C). The observed effects of photo-reduction highlight that care has to be taken when in-solution data of ferric proteins are rationalized by structural constraints derived from crystal structures of reduced enzymes.

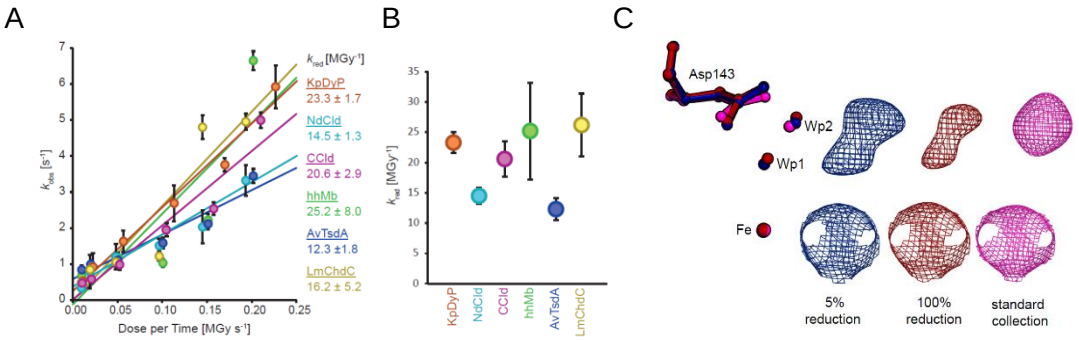

Figure 1: Reduction kinetics and their effects on iron ligation: (A) linear dependence of the observed reaction speed k_{obs} on the dose and (B) rate of reduction k_{red} of the six model proteins. (C) Electron density maps of the heme iron and active site waters show the effect of x-ray induced reduction.

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[2] T. Beitlich, K. Kühnel, C. Schulze-Briese, R. L. Shoeman, I. Schlichting, J Synchrotron Radiat **14** (2007), 11-23

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