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Keywords: radiation damage, swept volume, dose

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X-ray induced photoreduction of cobalamins

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Cobalamin cofactors crystallize very well and their crystals diffract to very high resolution. This makes them ideal model systems to study the mechanisms of (X-ray induced) photoreduction of redox sensitive corrine systems which play an important role in many metallo-proteins.

The photoreduction of several cobalamin cofactors in enzymes has been investigated extensively. The generally accepted mechanism in case of adenosylcobalamin is a homolytic cleavage of the Co(III)-C-bond going along with the formation of a 5'-deoxyadenosyl radical. [1], [2].

Radiation damage of biological samples is a major impediment to the sucIn our study we have further investigated the X-ray induced photoreduction of cobalamins by XANES and high resolution X-ray diffraction experiments. One mechanism proposed for X-ray photoreduction of metal organic compounds and metallo-proteins is X-ray induced water photolysis generating free electrons which then reduce the metal atom. [1],[3].

Our XANES measurements revealed that the presence or absence of water in the sample has only minor influence on the photoreduction. Lowering the temperature reduces the susceptibility to photoreduction of cyanocobalamin. This further indicates that no direct photoreduction by the photo electrons is taking place. In such a case a temperature independent susceptibility would be expected.

In our X-ray diffraction measurements of methylcobalamin and cyanocobalamin specific bond length changes as function of dose could be observed, which can be attributed to X-ray induced photoreduction. In case of cyanocobalamin, analysis of the thermal displacement parameters showed a strong B-factor increase for one specific hydrogen atom. This increase was observed in all of 6 independent measurements. Such an intramolecular hydrogen abstraction might be of importance for the X-ray induced photoreduction of cobalamins and could explain the observed temperature dependence.

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Kewords: cobalamin, photoreduction, specific radiation damage

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Phasing Selenomethionine proteins using UV induced radiation damage

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Selenium is the most widely used heavy atom for experimental phasing, either by single anomalous scattering (SAD) or multiple-wavelength anomalous dispersion (MAD) procedures. The use of the single isomorphous replacement (SIR) or single isomorphous replacement with anomalous scattering (SIRAS) phasing procedure with selenomethionine (Mse) containing proteins is not so commonly used, as it requires isomorphous native data.

Several non-redundant X-ray diffraction data sets from various Mse derivatised protein crystals were collected at energies far below the absorption edge before and after exposing the crystal to ultraviolet (UV) radiation with 266 nm lasers. A detailed analysis revealed that significant changes in diffracted intensities were induced by ultraviolet irradiation whilst retaining crystal isomorphism. These intensity changes allowed the crystal structures to be solved by the radiation-damage-induced phasing (RIP) technique [1]. These can be coupled with the anomalous signal from the dataset collected at the selenium absorption edge to obtain SIRAS phases in a UV-RIPAS phasing experiment [2].

Inspection of the crystal structures and electron-density maps demonstrated that covalent bonds between selenium and carbon at all sites located in the core of the proteins or in a hydrophobic environment were much more susceptible to UV radiation-induced cleavage than other bonds typically present in Mse proteins. The rapid UV radiation-induced bond cleavage opens a reliable new paradigm for phasing when no tunable X-ray source is available.

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Keywords: UVRIP, Mse, phasing

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Crystal structures of two archaeal Pelotas reveal inter-domain structural plasticity

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Dom34 from *Saccharomyces cerevisiae* is one of the key players in no-go mRNA decay, a surveillance pathway by which an abnormal mRNA stalled during translation is degraded by an endonucleolytic cleavage. Its homologs called Pelota are found in other species. We showed previously that *S. cerevisiae* Dom34 (domain 1) has an endoribonuclease activity, which suggests its direct catalytic role in no-go decay. Pelota from *Thermoplasma acidophilum* and Dom34 from *S. cerevisiae* have been structurally characterized, revealing a tripartite architecture with a significant difference in their overall conformations. To gain further insights into structural plasticity of the Pelota proteins, we have determined the crystal structures of two archaeal Pelotas from *Archaeoglobus fulgidus* and *Sulfolobus solfataricus*. Despite the structural similarity of their individual domains to those of *T. acidophilum* Pelota and *S. cerevisiae* Dom34, their overall conformations are distinct from those of *T. acidophilum*

Pelota and *S. cerevisiae* Dom34. Different overall conformations are due to conformational flexibility of the two linker regions between domains 1 and 2 and between domains 2 and 3. The observed inter-domain structural plasticity of Pelota proteins suggests that large conformational changes are essential for their functions [1].

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Keywords: conformational flexibility, no-go decay, ribonulease

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Structural studies on piRNA recognition by mammalian PIWI-like Argonaute proteins

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RNA silencing is a mechanism of gene regulation that affects virtually all biological processes. In combination with a family of proteins called the Argonautes (AGOs and PIWIs), small RNAs target larger nucleic acids (e.g. mRNAs) in a sequence-defined manner to turn down the expression of specific genes. Based on sequence analyses, different classes of small RNAs are enriched for specific nucleobases at their 5' end. The Argonaute MID domain anchors the 5`-phosphorylated end of the small RNA guide strand. This interaction is necessary for coupling small RNAs to one of the multiple Argonautes present in the cell, which can then perform its downstream effector activity. Our group recently discovered the molecular basis of human AGO2 selectivity for U and A at the 5'-end of micro RNAs (miRNAs). We showed that a short rigid loop with a specific conformation in the AGO2 MID domain confers this selectivity exclusively via protein backbone interactions [1]. Like miRNAs, PIWI-interacting RNAs (piRNAs) show a strong preference for U at the 5'-end of the guide RNA strand [2]. A question that arises from these observations is whether PIWIs can enforce the same type of selection on piRNAs via the identity of their 5'-nucleotides.

To answer this question, we determined the first crystal structures of MID domains from mammalian PIWI-like Argonautes. Although their structures show the same overall fold as human AGO2 there is a substantial difference in the positioning of the C-terminal alpha helix. This results in a loss of a critical lysine residue that is normally required to bind the phosphate group of the 5'-nucleotide in AGO-like Argonautes. Additionally, the nucleotide specificity loop in PIWI has a different conformation relative to that observed in AGO2 and suggests that side chains may also be involved in interacting with the base. The impact of these differences on interaction with piRNA will be studied using NMR titration experiments and functional assays on full-length PIWI-like Argonautes.

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Keywords: RNA silencing, Argonautes, MID domain

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Optimal fine φ slicing for single photon counting pixel detectors

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The data collection parameters used in a diffraction experiment have a strong impact on the quality of the acquired data. A careful choice of parameters leads to better data and can make the difference between success and failure in phasing attempts and better data will also result in a more accurate atomic model. The selection of data acquisition parameters has to account for the application of the data in various phasing methods or high-resolution refinement [1]. Furthermore, experimental factors like crystal characteristics, available experiment time, and the properties of X-ray source and detector have to be considered.

CCD detectors are for many years the prevalent type of detectors used in macromolecular crystallography. Most recommendations for data collection strategies as well as the experience of the experimenters are based on the characteristics of this detector type. Recently, hybrid pixel X-ray detectors that operate in single-photon-counting mode became available for macromolecular crystallography [2,3]. The commercially available PILATUS hybrid pixel detector is now in standard user operation at an increasing number of macromolecular crystallography synchrotron beamlines. Hybrid pixel detectors have fundamentally different characteristics and offer various advantages over CCD detectors [3,4]: (i) No readout noise and dark current. (ii) A sharp point spread function of one pixel. (iii) A short readout time in the millisecond range. (iv) A high dynamic range of 20 bits.

To fully exploit the advantages of hybrid pixel detectors different data collection strategies than those established for CCD detectors have to be applied because of the different characteristics of the two types of detectors. Fine φ slicing is a strategy particularly well suited for hybrid pixel detectors because of the fast readout time and the absence of readout noise. We systematically collected a large number of data sets from crystals of four different proteins to investigate the benefit of fine φ slicing on data quality with a noise-free detector in practice. Our results show that fine φ slicing can substantially improve scaling statistics and anomalous signal but potentially problems can arise when Δφ is only a small fraction of the crystal mosaicity.

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Cases of Twinning at the Joint Center for Structural Genomics

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Twinning has been observed in a number of structures at the Joint Center for Structural Genomics (JCSG). Eighteen examples have been refined and deposited in the PDB of which fourteen were solved by MAD and the rest by molecular replacement. Several other targets that had twinned crystals also had non-twinned crystal forms that were ultimately solved and deposited. The twins can be classified as