we determined that hydrogen gas, mainly originating from aliphatic C-H bonds, is a major cause of global radiation damage in protein crystals [1]. At cryogenic temperatures the gas remains inside the crystals causing lattice deformations by the generation of an inner pressure. This results in the well known decrease of diffraction power with increasing dose.

X-ray diffraction experiments, performed in order to directly identify the location of hydrogen abstraction, have been difficult. This is due to the small hydrogen X-ray scattering lengths. In an X-ray irradiation experiment on the polypeptide Cyclosporine A, we were able to indirectly observe hydrogen abstraction by X-ray induced bond lengths changes [2].

To further investigate this effect we have performed a combined synchrotron X-ray irradiation - neutron diffraction experiment. The results confirmed our findings from the previous X-ray diffractions experiments and further revealed that X-ray induced hydrogen abstraction is highly selective process.

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Keywords: radiochemistry, macromolecules, X-ray diffraction

MS.70.4

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Temperature and Time Dependent Studies of Radiation Damage

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Temperature-dependent X-ray crystallography has been used to characterize the time, space and energy dependence of radiation damage to protein crystals.

The sensitivity of global damage to protein crystals exhibits a dynamical transition near 200 K [1]. Below the transition, an activation energy for damage of ~1 kJ/mol, similar to that for solvent-free small molecule organic crystals, is observed, and may be associated with vibrationally assisted reactions. Above the transition, the activation energy of ~18 kJ/mol is similar to that for diffusive motions in the protein and solvent.

These diffusive motions continue after the X-rays have been shut off, and from 300 K to 180 K we observe "dark progression" of radiation damage. The rate of dark progression has an Arrhenius temperature dependence with an activation energy of 15 kJ/mol,and its timescale decreases from ~1000 s at 180 K to ~10 s at 300 K, suggesting the feasibility of outrunning radiation damage using faster data collecton. At intermediate temperatures (200-240 K), faster data collection does appreciably reduce radiation damage. But at room temperature, the timescales for the dominant diffusive damage processes are less than 2 s, and damage shows no dose rate dependence for dose rates between 8 and 300 kGy/s.

The spatial distribution of damage within the unit cell varies with temperature. At low temperatures, disulfide bridges and crystal contacts are readily damaged. But at 240 K and above, where diffusive motions are important and overall radiation sensitivity is much greater, solvent-exposed turns are the most sensitive while buried residues and residues involved in crystal contacts are more stable. These observations add detail to the common notion that damage at room temperature is due to diffusive motions.

Finally, 19 small molecule compounds, most known to be effective free-radical scavengers in solution, have been examined for possible protective effects in protein crystals. At room temperature, none significantly reduces radiation damage, and several increase it; at T=100 K, no protective or sensitizing effects are observed. Scavengers are ineffective in protecting protein crystals because a large fraction of the incident radiation is absorbed by protein atoms and because the ratio of scavenger molecules to protein molecules is too small to provide appreciable competitive protection.

This work was conducted at the MacCHESS facility at CHESS and in collaboration with IMCA-CAT at the APS.

[1] M. Warkentin, R.E. Thorne, *Acta Crystallographica Section D-Biological Crystallography* **2010,** *66*, 1092-1100.

Keywords: temperature, radiation, dynamics

MS.70.5

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Combining X-Ray Diffraction and *in-situ* Spectroscopy to Study Haem Proteins

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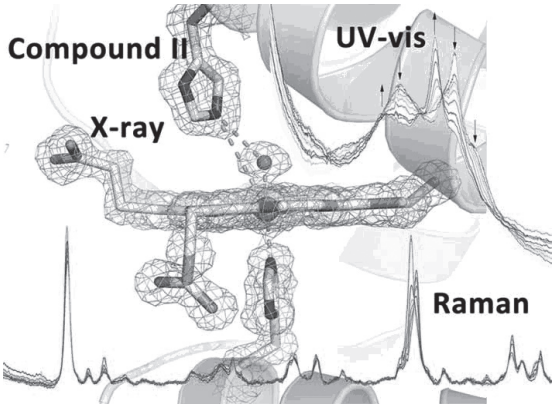
The influence of X-ray radiation damage to protein crystals is well known to occur even at cryogenic temperatures, and redox active sites like metal sites seem especially vulnerable for radiation-induced reduction. It is essential to correctly know the oxidation state of these metal sites in protein crystal structures, to be able to interpret the structure-function relation.

We have used *in-situ* (online) UV-vis and Raman spectroscopy to study how different oxidation states of the haem proteins myoglobin and catalase-peroxidase are influenced by X-rays during crystallographic data collection. The spectroscopic changes have been monitored as a function of X-ray exposure (dose absorbed), and show that the different redox-states in myoglobin vary in how fast they are "reduced" by the X-rays (e.g. ferric Fe^{3+} myoglobin is reduced faster than ferryl Fe^{IV}=O myoglobin) [1], and there is also differences between ferric myoglobin and catalase-peroxidase. The higher oxidation states of myoglobin are not reduced to normal ferrous Fe^{2+} or ferric Fe^{3+} states, but end up in some intermediate state. One of the primary goals of the project has been to characterise and study the different intermediates in the reaction between myoglobin and peroxides [2], [3]. The reaction intermediates generated in this reaction appear biologically relevant since myoglobin is proposed to function as a scavenger of reactive oxygen species during oxidative stress.



We have also been able to use the radiation damage to generate an

otherwise unstable and unattainable state by cryoradiolytic reduction of an oxymyoglobin equivalent (Compound III) to generate and trap the so-called peroxymyoglobin intermediate. By annealing this compound the oxygen-oxygen bond is broken and the reaction propagates to the ferryl compound II intermediate [3], [4].

[1] H.-P. Hersleth, K.K. Andersson, *Biochim. Biophys. Acta* **2011**, In press. DOI: 10.1016/j.bbapap.2010.07.019. [2] H.-P. Hersleth, *et al*., *J. Biol. Chem.* **2007**, *282*, 23372-23386. [3] H.-P. Hersleth, Y.-W. Hsiao, U. Ryde, C.H. Görbitz, K.K. Andersson, *Biochem. J*. **2008**, *412*, 257-264. [4] H.-P. Hersleth, Y.-W. Hsiao, U. Ryde, C.H. Görbitz, K.K. Andersson, *Chem. Biodiv*. **2008**, *5*, 2067-2089.

Keywords: haem proteins, radiation damage, spectroscopy

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Structural basis of double-stranded RNA recognition by RIG-I

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Innate immunity requires sensory molecules to detect pathogens. RIG-I-like receptors (RLRs: retinoic acid-inducible gene I, RIG-I; melanoma differentiation-associated gene 5, MDA5; and laboratory of genetics and physiology 2, LGP2) sense viral RNAs and result in immunological responses against viral infection. RLRs belong to a family of cytoplasmic DExD/H box RNA helicases. The helicase domain of RIG-I and MDA-5 is connected to two caspase activation and recruitment domains (CARDs) at the N terminus and a Zn ion binding regulatory domain at the C terminus**.** Upon binding and activation by viral dsRNA or triphosphated RNA, RIG-I and MDA-5 recruit the adaptor IPS-1 (also known as MAVS, CARDIF or VISA) on the outer membrane of the mitochondria through the CARDs domain. This leads to the activation of several transcription factors including IRF3, IRF7 and NF- κ B, and the production of type I interferon (IFN) and inflammatory cytokines.

Several crystal structures of the regulatory domains and their complexes with duplex RNA (dsRNA) are available, providing structural insights into RNA recognition by the RD domain. The role of the helicase domain in RNA sensing and CARDs activation is still largly unknown. To understand the mechanistic basis of RIG-I activation, we determined the crystal structure of RIG-I dsRNA complex. In this structure, the dsRNA interacts extensively with both the helicase domain and the regulatory domain, forming a "hotdog" like complex. The linker region between the two domains adopts a lever-like conformation, suggesting the coupling between the two domains upon activation by dsRNA. Within the helicase domain, Rec-A like domain 1 dominates the interaction with the dsRNA, and this interaction may be responsible for activation of the CARDs domain. The two Rec-A like domains adopt an open conformation in the absence of ATP. This suggests additional conformational changes may occur upon ATP hydrolysis, providing a means of switching a signal on and off, to allow tight regulation of the host immune response. Structural and biochemical studies of full length RIG-I will give more insights into the process of RIG-I activation.

To our knowledge, this is the first structure of a super-family 2 protein (SF2, which are RNA-dependent ATPases, and often helicases) in complex with duplex RNA. The structural and functional diversity of the "helicase" family is now expanded.

Keywords: RIG-I helicase, virus RNA, innate immunity

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Crystallographic insights into the structure of spliceosomal snRNPs

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The protein-coding regions of most eukaryotic genes are interrupted by non-protein-coding sequences called introns. The entire gene, including the introns, is transcribed as precursor mRNA (pre-mRNA) from which introns are removed and protein-coding regions ligated together by the spliceosome, a dynamic, multisubunit assembly [1]. The five splicesomal snRNPs (U1, U2, U4, U5 and U6) are its primary components. The snRNPs, along with many *trans*-acting protein factors, recognise the intron boundaries, catalyse intron excision and the subsequent ligation of the exons.

We have reconstituted snRNP particles and sub-particles, crystallized them and solved their structures in order to gain insight into spliceosomal snRNP structure.

The ten subunit, functional core of U1 snRNP was reconstituted from an *in vitro* transcribed RNA along with the seven Sm core proteins and the U1-specific U1-70k and U1-C proteins, all recombinantly expressed in *Escherichia coli*. The particle's crystal structure was solved at 5.5 Å resolution [2]. This was the first crystal structure of a spliceosomal snRNP. A striking feature is the N-terminal polypeptide of U1-70k, which extends over 180 Å from its RNA binding domain, wraps around the Sm protein core domain, and finally contacts the U1-C protein on the far side of the particle. The interaction of U1-C with the 5' end of U1 snRNA, which base-pairs with a putative 5' splice site mimic, suggests why U1-C is crucial for 5' splice-site recognition.

We have also assembled the U4 snRNP core domain. The RNA consists of the Sm site and two flanking hairpins, to which are bound the seven Sm proteins: B, D3, D1, D2, F, E and G. The Sm proteins form a heptameric ring through which passes the single-stranded Sm site. The crystal structure was solved at 3.6 Å resolution[3]. A hydrogen-bonding scheme, which explains the recognition and specificity of the Sm proteins for the Sm site is inferred from the structure and this is likely conserved in the U1, U2, U4 and U5 snRNPs. Comparison with the U1 structure suggests that although the core Sm binding site is recognised in a similar way by different snRNPs, there are differences in how the cores interact with other regions of the snRNPs.

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MS.71.3

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Structural studies of a CRISPR RNA processing endonuclease

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The CRISPRs (Clustered Regularly Interspaced Short Palindromic