Burkardt, M. Erbudak, J.N. Longchamp *Surf. Science* **2007,** *601*(2), 544-551. [3] D. Sutter-Widmer, S. Deloudi, W. Steurer, *Physical Review.* **2007,** B*75*, 094304. [4] D. Sutter-Widmer, S. Deloudi, W. Steurer, *Philosophical Magazine* **2007,** *87*(18-21), 3095-3102. [5] W. Steurer. S. Deloudi, *Crystallography of Quasicrystals: Concepts, Methods and Structures,* **2009**, vol. 126 of *Series in Materials Science*. Springer. [6] W. Steurer, T. Haibach, *Acta Crystallographica* **1999**, A*55*, 48–57.

Keywords: quasicrystal, tiling, periodic average structure

MS.70.1

Acta Cryst. (2011) A**67,** C158

Radiation Damage and Mn Metalloproteins

Junko Yano,[a] Jan Kern,[a] Vittal K. Yachandra,[a] James Whittaker,[b] [a]*Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, (US*A). [b]*Department of Science and Engineering, School of Medicine, Oregon Health and Science University, Beaverton, OR 97006, (US*A). E-mail: jyano@lbl.gov

Manganese complexes play critical roles in the metabolism of oxygen that is important in many biological systems. Among these systems, one of the most important is the photo-induced oxidation of water to dioxygen by the photosynthetic membrane protein complex, Photosystem II (PS II). This reaction is catalyzed by a Mn_4CaO_5 cluster located in the PS II membrane complex. One other system is a catalase that contains a binuclear Mn cluster, that disproportionates peroxide to water and dioxygen.

We have shown previously by X-ray spectroscopy that the Mn_4Ca cluster is highly susceptible to the X-ray radiation damage, particularly under the condition that the diffraction data have been commonly collected [1]. We have detailed XAS studies as a function of dose, temperature, energy, and time. We have also completed a similar X-ray damage study using XAS of the oxidized and reduced Mn catalase.

Recently, the crystal structure of PS II isolated from thermophile was reported at a resolution of 1.9 A [2] by collecting the data at much lower X-ray dose than that has been used for the earlier PSII crystallography studies. The electron density map clearly shows the geometry of the four metals and one Ca. Their study for the first time gives us a starting point to think about the detailed chemical structure of the Mn_4CaO_5 cluster in the dark state (S_1) and also the consequence of specific radiation damage to the redox-active Mn site. The crystal structures of the Mn catalase have also been reported with high resolutions (~ 1 A) [3,4].

We have compared the effect of X-ray radiation damage on the two major Mn metalloprotains, PS II and Mn catalase. We discuss possible differences between these two cases. The study also gives us an insight into the unique effect of radiation damage to individual metalloproteins and the importance of the combination of the spectroscopic techniques and crystallography in order to obtain intact forms of the catalytic complexes [1,5].

[1] J. Yano, J. Kern, K.D. Irrgang, M.J. Latimer, U. Bergmann, P. Glatzel, Y. Pushkar, J. Biesiadka, B. Loll, K. Sauer, J. Messinger, A. Zouni, V.K. Yachandra, *Proc. Natl. Acad. Sci. USA.* **2005,** *102*, 12047-12052. [2] Y. Umena, K. Kawakami, J.R. Shen, N. Kamiya, *Nature* **2011**, *in press*. [3] S.V. Antonyuk, W.R. Melik-Adamyan, A.N. Popov, V.S. Lamzin, P.D. Hepstread, P.M. Harrison, P.J. Artymiuk, V.V. Barynin, *Cryst. Reports* **2000,** *45*, 11-22. [4] V.V. Barynin, M.M. Whittaker, S.V. Antonyuk, V.S. Lamzin, P.M. Harrison, P.J. Artymiuk, J.W. Whittaker, *Structure* **2001,** *9*, 725-738. [5] T.L. Stemmler, T.R. Sossong, D.E. Ash, T. Elgren, D. Kurtz, J.E. Penner-Hahn, *Biochemistry*, **1997**, *36*, 9847-9858.

Keywords: photosynthesis, manganese, xanes

MS.70.2

Acta Cryst. (2011) A**67,** C158

Radiation Damage to Protein Crystals is Reduced with a Micron-sized X-ray Beam

Robert F. Fischetti,[a] Ruslan Sanishvili,[a] Derek Yoder,[a] Sudhir Pothineni,[a] Gerd Rosenbaum,[b] Shenglan Xu,[a] Oleg Makarov,[a] Sergey Stepanov,[a] Venugopalan Nagarajan,[a] Stefan Vogt,[c] J. L. Smith,[a,d] [a]*GM/CA CAT and Biosciences Division, Argonne National Laboratory, Argonne, IL.* [b]*Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA.* [c]*X-Ray Science Division, Argonne National Laboratory, Argonne, IL.* [d]*Life Sciences Institute, Department of Biological Chemistry, University of Michigan, Ann Arbor, MI (USA).* E-mail: rfischetti@anl.gov.

The small, intense X-ray beams available at 3rd generation light sources have been exploited by structural biologists to determine the structure of increasing larger or more complex macromolecules. The crystals of many of these macromolecules may have a largest dimension of only 5-10 microns, and may diffract poorly due to lack of internal order. Obtaining data of high signal-to-noise requires exposing the crystal to a beam of high flux density, resulting in increased absorbed dose and radiation damage. Although cryo-cooling of protein crystals significantly reduces X-ray induced radiation damage, it does not eliminate the damage.

The predominant mechanism of interaction of an X-ray with a low-Z atom in the crystal is the emission of a photoelectron, which carries away most of the energy of the incident X-ray. When the emitted photoelectron scatters off another atom, it loses energy to the atom resulting in local damage. As the photoelectron energy decreases, the probability of interacting with yet another atom increases causing more frequent interactions until finally the photoelectron is recaptured. Thus, if the X-ray beam size is small compared to the distance the photoelectron travels from its point of emission, then deposition of photoelectron energy outside the beam footprint may reduce radiation damage inside the beam footprint. Monte-Carlo simulations predict that a photoelectron of typical energy could travel 4 – 5 μm from the point of emission before being absorbed. We studied radiation damage to lysozyme crystals by monitoring the diffracted intensity of 18.5-keV X-rays as a function of dose and beam size (0.86 – 15.6 μm) at beamline 23-ID-B at the Advanced Photon Source. We observed a 3-fold reduction of damage per dose within the footprint of the smallest compared to the largest beam. In addition, the spatial extent of radiation damage was mapped using both 15.1- and 18.5-keV X-rays and a ~1-μm beam. The damage profiles displayed spatial anisotropy with greater damage occurring along the direction of the X-ray polarization, as expected. The spatial extent of the damage was limited to about 4 μm.

GM/CA CAT is supported by the NIH National Institute of General Medical Sciences and National Cancer Institute. The APS is supported by the US Department of Energy.

Keywords: radiation, damage, microcrystallography

MS.70.3

Acta Cryst. (2011) A**67,** C158-C159

The Role of Hydrogen in Radiation Damage

Alke Meents,[a] Armin Wagner,[b] Matthias Gutmann,[c] Desiree Heintz,[a] Anja Burkhardt,[a] [a]*Deutsches Elektronen Synchrotron DESY, Hamburg, (Germany).* [b]*Diamond Light Source, Oxfordshire, (UK).* [c]*Rutherford Appleton Laboratory, ISIS, Oxfordshire, (UK).* E-mail: alke.meents@desy.de

Radiation damage of biological samples is a major impediment to the success of experiments using ionizing radiation. In a recent study

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.

[1] A. Meents, S. Gutmann, A. Wagner, C. Schulze-Briese. *PNAS* **2010,** *107,* 1094-1099. [2] A. Meents, B. Dittrich, S. Gutmann. *J. Synchrotron Rad.* **2009,** *16,* 183-190.

Keywords: radiochemistry, macromolecules, X-ray diffraction

MS.70.4

Acta Cryst. (2011) A**67,** C159

Temperature and Time Dependent Studies of Radiation Damage

Robert E. Thorne,[a] Matthew Warkentin,[a] Jan Kmetko,[b] Ryan Badeau,[a] Jesse B. Hopkins,[a] *[a]Departament of Physics, Cornell University, Ithaca, NY, (USA). [b]Department of Physics, Kenyon College, Gambier, OH, (USA).* E-mail: ret6@cornell.edu

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

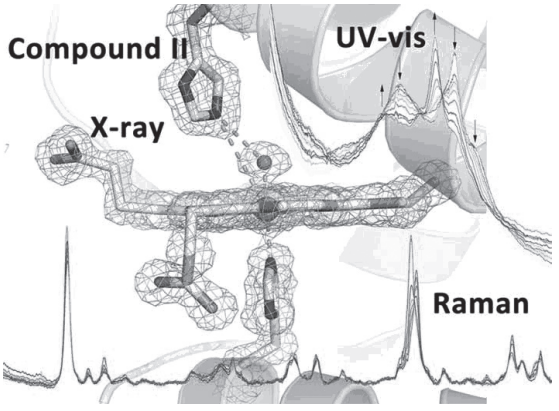
Acta Cryst. (2011) A**67,** C159-C160

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

Hans-Petter Hersleth,[a] Xiangbo Zhao,[b] Richard S. Magliozzo,[b] K. Kristoffer Andersson,[a] *[a]Departament of Molecular Biosciences, University of Oslo (Norway). [b]Department of Chemistry, Brooklyn College, New York (USA).* E-mail: h.p.hersleth@imbv.uio.no

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