11th International Workshop on X-ray Radiation Damage to Biological Samples

Abstracts

Contents

Schedule

Day1: Wednesday, 14th October, 2020

13:00	**Welcome Message** Elspeth Garman
Session 2: Practical Aspects of Reducing Radiation Damage at Synchrotrons Chair: Ana Gonzalez (MAXIV)	
13:20	**Alexander Popov** DOZOR, MESH-BEST and 3D-BEST: software for data collection optimization taking into account radiation damage
13:50	**Kunio Hirata** Effects of dose control in multiple small wedge data collection
Session 1: Biological Studies affected by Radiation Damage Chairs: Elspeth Garman (Oxford) , Martin Fuchs (NSLSII)	
14:20	**Michi Suga** X-ray free electron lasers reveal the molecular mechanism for water oxidation in photosystem II
14:50	**Kristina Carugo** X-ray induced radiation damaged on metal centers in proteins
15:20	Tea and Posters
15:40	**Robin Owen** Approaches for following X-ray induced reactions in crystallo
16:10	**Mike Hough** Room Temperature Serial Crystallography of Peroxidases at Synchrotron and XFEL beamlines
16:40	**Corie Ralston** X-ray footprinting of proteins: using hydroxyl radical damage to infer structural conformation
17:10	**A word from our Sponsors**
17:25	**Open Discussion** Chairs: Ian Carmichael, Florian Dworkowski

Day2: Thursday, 15th October, 2020

Session 3: Damage at New Sources - XFEL and 4th generation synchrotrons Chairs: Karol Nass (SwissFEL), Ilme Schlichting (MPI Heidelberg)	
13:00	**Nils Huse** UV-induced cleavage and geminate recombination of the disulfide bond motif followed via ultrafast X-ray absorption spectroscopy
13:30	**Alexander Gorel** Structural dynamics in proteins induced by and probed with X-ray free-electron laser pulses
14:00	**Antoine Royant** Specific radiation damage is a lesser concern at room temperature
14:30	**Nicolas Coquelle** Radiation damage in serial synchrotron crystallography at cryo- and room temperature
15:00	**Marie Luise Gruenbein** Avoiding multiphoton artefacts in time-resolved pump probe experiments
15:30	**Poster Blitz Presentations**
15:50	Tea and Posters
16:10	**Carl Caleman** Is radiation damage the limiting factor in high-resolution single particle imaging with X-ray free-electron lasers?
16:40	**Yulia Pushkar** X-ray Emission Spectroscopy at X-ray Free Electron Lasers: Limits to Observation of Unperturbed Electronic Structures.
17:10	**James Holton** Correcting Non-isomorphism
17:40	**Open Discussion** Chairs: Arwen Pearson, Masaki Yamamoto

Day3: Friday, 16th October, 2020

Session 4: Radiation Damage in Complementary Fields including Biological Imaging Chair: Raimond Ravelli (U of Maastricht)	
13:00	**Joshua Dickerson** Dose calculations for microcrystallography, XFELs, and electron microscopy: extensions to RADDOSE-3D
13:30	**Robin Santra** Quantitative simulation tools for predicting radiation damage driven by high-intensity x-ray pulses
14:00	**Pieter Glatzel** Radiation damage in X-ray spectroscopy
14:30	**Breakout Room Discussions**
15:20	Tea and Posters
15:40	**Yunyun Gao** An Objective Metric for Correcting Radiation Damage in SAXS
16:10	**Maciej Kozak** Domain swapping in solution induced by irradiation – case study human cystatin C and other proteins
16:40	**General Discussion and Farewell** Chairs: Vincent Olieric, Martin Weik

General Information

Sponsors

We kindly thank our sponsors:

Scientific Committee

Elspeth Garman (Oxford, UK)
Martin Weik (IBS, France)
Ian Carmichael (Notre Dame Radiation Laboratory, USA)
Florian Dworkowski (PSI, Switzerland)
Martin Fuchs (BNL, USA)
Ana Gonzalez (MAX IV, Sweden)
Max Nanao (ESRF, France)
Karol Nass (PSI, Switzerland)
Colin Nave (DLS, U.K.)
Vincent Olieric (PSI, Switzerland)
Arwen Pearson (University of Hamburg, Germany)
Raimond Ravelli (Maastricht University, The Netherlands)
Gerd Rosenbaum (University of Georgia, USA)
Ilme Schlichting (MPI Heidelberg, Germany)
Masaki Yamamoto (SPring8, Japan)

Local Organizers

Florian Dworkowski
Karol Nass
Vincent Olieric

Sonia Reber (Secretariat)
Stefan Müller (SLS TT AG)

Contact

Email: rd11@psi.ch
Conference Phone: +41 (0)79 387 05 42

Previous Workshops

RD 1	*1999*	*ESRF, Grenoble, France*
RD 2	*2001*	*APS, Chicago, USA*
RD 3	*2003*	*ESRF, Grenoble, France*
RD 4	*2006*	*SPring-8, Sayo, Japan*
RD 5	*2008*	*PSI SLS, Villigen, Switzerland*
RD 6	*2010*	*SSRL, Stanford, USA*
RD 7	*2012*	*DLS, Didcot, UK*
RD 8	*2014*	*EMBL & DESY, Hamburg, Germany*
RD 9	*2016*	*MAXIV, Lund, Sweden*
RD 10	*2018*	*NSLSII, Brookhaven, USA*
RD 11	*2020*	*Virtual*

Speaker Abstracts

DOZOR, MESH-BEST and 3D-BEST: software for data collection optimization taking into account radiation damage

Alexander Popov[1],
Gleb Bourenkov[2], Igor Melnikov[1]

[1] ESRF, France
[2] EMBL-Hamburg, Germany

apopov@esrf.fr

Success of crystallographic experiments using weakly diffracting macromolecular crystals depends on a proper choice of the data collection protocol. Radiation damage is a critical factor limiting data quality and being able to predict the absorbed dose and mitigate the effects of exposure to X-rays is very important.

We present the set of methods and programs intended for optimal planning of X-ray diffraction by accounting the experimental purpose, the characteristics of the X-ray beam and instrumentation and by the samples properties. The information about the crystals mounted in the sample holder is taken from low-dose two-dimensional raster X-ray diffraction scans. The image analysis is carried out by program Dozor which recognizes the presence of a diffraction pattern from a macromolecular crystal, estimates the diffraction signal and produces the list of diffraction spot positions. Bases on Dozor's output, the program MeshBest determines the areas of the mesh-scans which belong to the individual crystals and produces a three-dimensional crystal map showing estimates of the dimensions, centre positions and diffraction qualities of each crystal contained in the holder. Using this map, the best achievable results of data collection for any individual crystals are estimated by software program 3D-BEST. In last version of BEST, the strategy-determination method takes into account the variations in irradiated crystal volume at the rotation and possible translation of the sample. Diffraction intensities at any moment of data collection is calculated as the sum of diffraction intensities from small crystal voxels taking into account the profile of primary X-ray beam and the dose absorbed by each voxel. Such modelling significantly improves the accuracy of predicted data statistics and allows selecting the best trajectory of the crystal movements for helical or for multi-positional strategy of data collection.

Effects of dose control in multiple small wedge data collection

Kunio Hirata[1],
Seiki Baba[2], Nobuhiro Mizuno[2], Naoki Sakai[1], Masaki Yamamoto[1]

[1] RIKEN/SPring-8 Center, Japan
[2] JASRI/SPring-8, Japan

kunio.hirata@riken.jp

Recent brilliant micro-focused X-ray has enabled high resolution structural analyses of challenging biological proteins such from 10-µm sized crystals. One of the key technologies to achieve this is merging many partial datasets. This so-called 'multiple small wedge data collection' strategy was initially developed at Advanced Photons Source to accomplish data collection from in-meso phase crystals of membrane proteins. The loop containing several tens of tiny LCP crystals is raster scanned by low dose micro-focused beam. Based on this scan, crystals embedded in lipids invisible under the visible light are aligned to X-rays. From the angle where raster scan is applied, small wedge dataset is collected from each found crystal. Finally, many wedges from many crystals are merged into the final dataset.

At SPring-8, we have achieved to determine many membrane protein structures in this strategy. Recently, we developed ZOO system which enables un-attended fully automated data collection. The system has accelerated data collection in multiple small wedge scheme. A function to estimate absorbed dose for data collection is equipped to the system. Our recommended absorbed dose for all data collection scheme is 10 MGy under a cryo condition. It is based on our knowledge-based parameter in study of radiation damage by using micro-focused beam. However, no body have conducted the systematic study of effect of accumulated absorbed dose in 'multiple small wedge' strategy. In the full rotation data collection using a single crystal without any translation of irradiation points, radiation damage heterogeneously accumulated on the crystal volume and effects to errors in intensities in the identical dataset. On the other hand, multiple small wedge data collection is based on merging many datasets. In this case, merging equilibrates the radiation damage because heavily damaged data region is slightly compensated by another crystal with freshness against to the damage.

We systematically investigated effects of dose control in multiple small wedge data collection by using several standard protein samples. We will share our results to have discussions about the best dose for small wedge data collection.

X-ray free electron lasers reveal the molecular mechanism for water oxidation in photosystem II

Michi Suga[1], Jian-Ren Shen[2]

[1] Okayama University, Japan
[2] JST PREST, Japan

Photosynthetic water oxidation is catalyzed by the Mn_4CaO_5-cluster [1,2] of Photosystem II (PSII) through a linear four oxidation intermediates of Si-state cycle (Si, i = 0-4). The catalyst becomes a Mn_4CaO_6-cluster in the S3-state by incorporation of additional oxygen O6 nearby a unique central oxo-bridge O53, supporting a dioxygen formation mechanism between O5 and O6. While insertion of the O6 has gradually been accepted, the chemical structure of O5 and O6 remain controversial so that several possible mechanisms for the O=O bond formation have been under debate.

To reveal the molecular details in the water oxidation reaction, we analyzed the X-ray free laser (XFEL) structures of PSII in the S1, S2 and S3 states by using fixed-target serial femtosecond crystallography with an XFEL provided by the SACLA [4]. In brief, single-shot diffraction images were collected in a fixed-data collection manner at a cryogenic temperature, in which PSII microcrystals were evenly sprayed on a mesh. Compared with serial femtosecond crystallography of PSII using a grease matrix as the injection medium at room temperature [3], this method reduced the sample consumption by one order of magnitude and ensured low background images, allowing us to analyze multiple datasets at 2.15-Å resolutions.

No insertion of water was found in the S2 state, but upon transition to the S3 state, flipping of Glu-189 provides a space for incorporation of the additional oxygen O6, and the Mn_4CaO_5-cluster remains in the open-cubane form. To determine the exact chemical structure of the O5 and O6, we examined four possible chemical species; superoxo, peroxo, oxyl/oxo, and oxyl/hydroxo. By altering the O5-O6 distance and examining the residual densities in the F_{obs}–F_{cal} difference Fourier map, we found that a distance of 1.9 Å resulted in the weakest residual densities. This suggests an oxyl/oxo coupling mechanism for the O=O bond formation in OEC. Moreover, the flipping of the Glu-189 also induces van der Waals repulsion between the carbonyl oxygen of Glu-189 and Ala-411CP43, which is transmitted to a strictly conserved short loop of the CP43 subunit that restricts the size of the O1-water channel. These structural changes in PSII between the different S-states reveal the mechanism of photosynthetic water oxidation by the cooperative action of substrate water access, proton release, and O=O bond formation.

Acknowledgments
We thank many collaborators who are not listed here due to the limited space.

References
[1] Umena, Y. et. al. Nature 473, 55-60, (2011).
[2] Suga, M. et al. Nature 517, 99-103, (2015).
[3] Suga, M. et al. Nature 543, 131-135, (2017).
[4] Suga, M. et al. Science 366, 334-338, (2019).

X-ray induced radiation damage on metal centers in proteins

Kristina Djinovic-Carugo

Department of Structural and Computational Biology, Max Perutz Labs, University of Vienna, Austria

kristina.djinovic@univie.ac.at

About one quarter to one third of all proteins are estimated to require metals to carry out their functions. Metalloproteins carry our diverse functions in cells from metal storage, to catalysis and signal transduction. In order to understand the chemistry underlying their function, high accurate structures are essential, and X-ray crystallography has been the most fruitful method for structure generation at high resolution. One of the first events taking place when crystals of metalloproteins are exposed to X-ray radiation is photoreduction of the redox-sensitive metal centres. Using standard experimental design for data-collection on high brilliance synchrotron sources will thus inevitably yield structure with reduced metal centre. Change of the metal oxidation state leads to alteration of the coordination stereochemistry, which can in in turn lead to misinterpretation of structure-function relationships, since biochemical and molecular biophysics data had been generated on a non-exposed and thus non-reduced sample in solution. Special care needs to be therefore taken in design of collection of metalloprotein X-ray diffraction data, keeping in mind that radiation damage will impact on the extracted biological information. Representative examples of X-ray induced metal photoreduction will be presented together with successful and failed examples of how to mitigate the damage.

Approaches for following X-ray induced reactions in crystallo

Robin Owen[1],
Danny Axford[1], Ali Ebrahim[1,2], Selina Storm[1], Hiroshi Sugimoto[3], Kensuke Tono[3],Shigeki Owada[3], Tadeo Moreno-Chicano[2], Richard Strange[2], Jonathan Worrall[2], Ivo Tews[4] & Michael Hough[2]

[1] Diamond Light Source, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE, UK
[2] School of life Sciences, University of Essex, Wivenhoe Park, Colchester, Essex, CO4 3SQ, UK
[3] Spring8/SACLA, Harima Campus, Hyogo, Japan.
[4] Biological Sciences, University of Southampton, Institute for Life Sciences, Southampton, SO17 1 BJ, UK

robin.owen@diamond.ac.uk

The development of serial crystallography has been driven by XFELs but is now firmly established at high-brilliance synchrotron sources. Serial crystallography provides a means of efficiently collecting diffraction data from crystals held at ambient temperatures, providing access to more physiological environments and helping make structural transitions directly observable. Data collection at room temperature comes, however, with a price in the form of the rapid onset of radiation damage and greatly reduced crystal lifetimes. While this drawback can be addressed in part through the use of serial approaches, significant challenges for the experimenter remain.

I will describe fixed target serial delivery methods developed and implemented at SACLA and Diamond, illustrating the gains that can be realised using a multi-faceted approach at different, complementary, sources as well as some of the challenges both met and remaining.

Room Temperature Serial Crystallography of Peroxidases at Synchrotron and XFEL beamlines

Mike Hough[1],
Ali Ebrahim[1,2], Marina Lucic[1], Tadeo Moreno-Chicano[1], Hiroshi Sugimoto[3], Kensuke Tono[3],Shigeki Owada[3], Danny Axford[4], Richard Strange[2], Jonathan Worrall[2] & Robin Owen[4]

[1] University of Essex, UK
[2] School of life Sciences, University of Essex, UK
[3] Spring8/SACLA, Harima Campus, Japan
[4] Diamond Light Source, Harwell Science and Innovation Campus, UK

mahough@essex.ac.uk

Metalloproteins, particularly those containing transition metals in high valent states are highly sensi-tive to redox state changes as a consequence of X-ray exposure during data collection. If not un-derstood and interpreted correctly, this site-specific damage may lead to misinterpretation of struc-tural data and mis-assignment of structures to e.g. enzyme mechanistic states. Increasing attention has been paid to room temperature crystallography to allow for protein dynamic freedom and in order to facilitate time-resolved crystallography. The binding of ligands to proteins may be affected both by temperature and redox state. The faster rate of radiation damage with dose in room temper-ature crystals poses challenges for data collection methodologies. I will describe room temperature fixed target 'chip' serial synchrotron crystallography (SSX) measurements at Diamond beamline I24 and serial femtosecond crystallography (SFX) experiments at the SACLA X-ray Free Electron Laser (XFEL), Japan. This system may be implemented with few changes at either synchrotron mi-crofocus or XFEL beamlines allowing us to directly compare the effects of radiation damage in SSX structures to XFEL structures collected using a near identical strategy i.e. where the nature of the X-ray beam is the primary experimental variable. I will describe applications of this approach to heme peroxidase systems [1-3], characterizing X-ray induced changes to the heme active sites and exploring the feasibility of ligand binding studies by SFX.

References

[1] Serial Femtosecond Zero Dose Crystallography Captures a Water-Free Distal Heme Site in a Dye-Decolorising Peroxidase to Reveal a Catalytic Role for an Arginine in FeIV=O Formation (2020) Angewandte Chimie Int. Ed. In press.
[2] Dose-resolved serial synchrotron and XFEL structures of radiation-sensitive metalloproteins. Ebrahim, A. et al., (2018) IUCrJ 6, 543-551.
[3] High-throughput structures of protein–ligand complexes at room temperature using serial femtosecond crystallography. Moreno-Chicano, T., et al. (2019) IUCrJ 6, 1074-1085.

X-ray footprinting of proteins: using hydroxyl radical damage to infer structural conformation

Corie Ralston

Lawrence Berkeley National Laboratory, USA

cyralston@lbl.gov

The method of X-ray footprinting mass spectrometry (XFMS) is used to investigate structural features and conformational changes of macromolecules in the solution state. XFMS is an in situ hydroxyl radical (•OH) labeling method; X-ray irradiation dissociates solvent water to produce hydroxyl radicals, which covalently modify side chains which are solvent accessible. More specifically, residues which are in proximity to water molecules (either bulk or bound) are modified to a greater extent than residues which are not in proximity to water. Because liquid chromatography-mass spectrometry is then used to analyze the stable covalent modifications produced, the data provide a "water map" at the single residue level, which is then used to determine sample conformation. In this talk, I will describe the XFMS method, its advantages and disadvantages relative to other methods, some recent examples of structural information obtained on protein systems using the method, and some recent instrumentation advances and plans for future improvements to the method.

Avoiding multiphoton artefacts in time-resolved pump probe experiments

Nils Huse

University of Hamburg, Hamburg, Germany

nils.huse@uni-hamburg.de

We investigated photocleavage of the disulfide bond motif in model compounds such as cysteine dimer (L-cystine) by time-resolved X-ray absorption spectroscopy [1,2]. We follow changes in X-ray absorption at the sulfur K-edge (2.5 keV) that appear to be unique to thyil (R-S·) radicals, thereby tracking the fate of the disulfide bond. Ultrafast spectroscopy has revealed homolytic S-S bond cleavage upon ultrafast 267-nm excitation [3], with a dominant yield of geminate recombination in solution within the first picosecond. A minor fraction of the thyil radicals survives at least for microseconds, reminiscent of X-ray induced radical formation [4]. Doubling the ultraviolet excitation energy yields new photoproducts, which indicate C-S bond cleavage. Weak relaxation of thyil charge density favors geminate recombination, suggesting at a natural inhibition of radiation from mid-ultraviolet excitation.

References

[1] M. Ochmann et al., J. Am. Chem. Soc. 139, 4797 (2017)
[2] M. Ochmann et al., J. Am. Chem. Soc. 140, 6554 (2018)
[3] K. Schnorr et al., J. Phys. Chem. Lett. 10, 1382 (2019)
[4] E. Y. Sneeden et al., J. Am. Chem. Soc. 139, 11519 (2017)

Structural dynamics in proteins induced by and probed with X-ray free-electron laser pulses

Alexander Gorel

Max-Planck-Institute for Medical Research, Heidelberg, Germany

alexander.gorel@mpimf-heidelberg.mpg.de

X-ray free-electron lasers (XFELs) enable crystallographic structure determination beyond the limitations imposed upon synchrotron measurements by radiation damage. The need for very short XFEL pulses can be relieved through gating of Bragg diffraction by loss of crystalline order as damage progresses but not if ionization events are spatially non-uniform due to underlying elemental distributions, as in biological samples. Indeed, correlated movements of iron and sulphur ions were observed in XFEL-irradiated ferredoxin microcrystals using unusually long pulses of 80 fs. Here we report a femtosecond time-resolved X-ray pump/X-ray probe experiment on protein nanocrystals. We observe changes in the protein backbone and aromatic residues as well as disulphide bridges. Simulations show that the latter's correlated structural dynamics are much slower than expected for the predicted high atomic charge states due to the significant impact of ion caging and plasma electron screening. This indicates that dense-environment effects can strongly affect local radiation damage-induced structural dynamics.

Specific radiation damage is a lesser concern at room temperature

Antoine Royant[1],
David von Stetten[1], Guillaume Gotthard[2], Daniele de Sanctis[1],
Sylvain Aumonier[1], Damien Clavel[3], Gordon Leonard[1]

[1] ESRF, Grenoble, France
[2] Paul Scherrer Institut, Villigen PSI, Switzerland
[3] Institut de Biologie Structurale, Grenoble, France

antoine.royant@esrf.fr

There are two types of radiation damage in protein X-ray crystallography [1]. The first one, global damage, has been known since the beginning of X-ray crystallography. Global damage accounts for the decrease in the diffraction properties of a crystal during data collection due to the interaction of X-rays with the atoms of the crystal, which, in particular, generates free electrons and radicals in the bulk solvent, progressively destroying the crystalline order. Global damage is slowed down by roughly two orders of magnitude on the dose scale when the diffraction experiment is performed at cryogenic rather than at room temperature. Cryo-crystallography has led to the explosion in the number of crystallographic protein structures in the 1990's as it allows determining a structure from a single crystal. However, it was then realized that a second type of radiation damage was at play in cryogenic experiments: specific damage. This damage affects certain specific chemical groups that are sensitive to electrons, for instance disulphide bonds, carboxylate groups or metal cations, which can be found in protein active sites. This can be explained by the fact that X-ray induced free electrons can still diffuse at cryogenic temperature. Therefore, specific damage may lead to artefacts in structural analysis of reaction intermediate states and thus in mechanistic interpretation. The discernibility of specific damage at cryogenic temperature means that there is a significant difference between the rates of the two types of damage, i.e. a 'decoupling' between the two phenomena. As room temperature protein crystallography is quickly developing thanks to the development of faster, noiseless detectors, of improved sample environment at room temperature and of the concept of serial crystallography, the question of the comparison of the respective rates of specific and global damage build-up at room temperature has become a hot topic. We have compared the rates of both damage build-up at cryogenic and room temperature for various proteins, including the reaction intermediate state of a fragment of a photoreceptor [2]. While the two types of damage are largely decoupled at cryogenic temperature (decoupling factor between 12 and 1600), they occur on a similar dose scale at room temperature (decoupling factor between 1 and 8). This indicates that depending on the studied protein, specific damage may not be a primary concern in crystallographic structure determination at room temperature, provided diffraction data can be collected from a single crystal. This should stimulate the development of time-resolved crystallography experiments at synchrotrons.

References

[1] Holton (2009) 'A beginner's guide to radiation damage' J. Synchrotron Rad. 16, 133–142.
[2] Gotthard et al. (2019). 'Specific radiation damage is a lesser concern at room temperature' IUCrJ, 6, 665-680.

Radiation damage in serial synchrotron crystallography at cryo- and room temperatures

Nicolas Coquelle[1,2],
Eugenio de la Mora[1], Charles S. Bury[3], Martin Rosenthal[4], James Holton[5,6,7], Ian Carmichael[8], Elspeth F. Garman[3], Manfred Burghammer[4], Jacques-Philippe Colletier[1], Martin Weik[1]

[1] University Grenoble Alpes, CEA, CNRS, Institut de Biologie Structurale, Grenoble, France
[2] Institut Laue Langevin, Grenoble, France
[3] Department of Biochemistry, University of Oxford, Oxford, UK
[4] European Synchrotron Radiation Facility, Grenoble, France
[5] Department of Biochemistry and Biophysics, University of California, San Francisco, USA
[6] Divison of Molecular Biophysics and Bioengineering, Lawrence Berkeley National Laboratory, Berkeley, USA
[7] Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, USA
[8] Notre Dame Radiation Laboratory, University of Notre Dame, Notre Dame, USA

coquelleni@ill.fr

X-ray crystallography is the most-prolific technique in structural biology but suffers from radiation damage, which limits the accuracy of the macromolecular structures. The introduction of cryo-cooling techniques greatly reduced the global radiation damage rate and was standardized on all X-ray crystallography beamlines at synchrotrons over the past decades. With the recent advent of serial crystallography, room temperature (RT) data collection was made more accessible as the absorbed energy is spread over a large number of crystals. So far, studies of specific and global radiation damage at RT still remain limited. Here, we used a sequential serial raster-scanning approach using the micro-focused beam of ESRF ID13 beamline in combination with a fast single-photon-counting pixel-array detector. Two series of 40 and 90 data sets were collected on Hen Egg-White Lysozyme (HEWL) crystals at RT and 100K, at resolutions of 2 and 1.9 Å, respectively. At RT, specific radiation damage was observed at disulfide bonds, but not on the carboxylic groups of acidic residues. The evolution of the specific damage at RT was monitored, and after an increase of damage with dose, its signal fades away. This peculiar behavior could be explained by differential diffraction intensity decay due to the non-uniform illumination by the X-ray beam and well modelled. Appearance of specific damage at RT is extremely fast and proceeds at a ~5-fold higher rate than global damage. Our results suggest it is advisable not to exceed about 0.4 MGy in static and time-resolved serial and oscillation synchrotron crystallography experiments at RT, a rough yardstick that will change for proteins other than HEWL and at resolutions other than 2 Å.

References

[1] de la Mora E, Coquelle N, Bury CS, Rosenthal M, Holton JM, Carmichael I, Garman EF, Burghammer M, Colletier J-P, Weik M (2020) Radiation damage and dose limits in serial synchrotron crystallography at cryo- and room temperatures. Proceedings of the National Academy of Sciences: 201821522

Avoiding multiphoton artefacts in time-resolved pump probe experiments

Marie Luise Gruenbein

Max-Planck-Institute for Medical Research, Heidelberg, Germany

marie.gruenbein@mpimf-heidelberg.mpg.de

Light is important for organisms from all domains of life, serving as an energy resource or carrier of information initiating intra- or intercellular signaling. Photosensitive proteins, endowed with a light-absorbing chromophore, enable this. Obtaining direct structural information to understand the underlying molecular mechanisms is not only important for the fundamental understanding of light-driven processes but has practical impact on future developments of e.g. fluorescent proteins for optical nanoscopy or retinal proteins for optogenetics.

For time-resolved crystallography small crystals that can be efficiently photolyzed are required. Serial data collection by means of free-jet sample injection is a convenient way for time-resolved pump probe experiments. In previous experiments employing this technique both at X-ray free-electron laser (XFEL) and synchrotron sources very high pump laser fluences ($\gg$ 100 $\mu J/mm^2$) have been used for optical excitation, a regime where multiphoton absorption of the chromophore is highly likely. However, multiphoton excitation differs significantly from the biologically relevant single photon absorption regime and generally results in different effects. This severely compromises the mechanistic interpretation.

We discuss this issue and present how to determine the appropriate photoexcitation intensity for time-resolved pump probe experiments. We discuss how to derive the required pump fluence based on the intensity distribution within the crystals and the jet used for delivering them into the X-ray beam and quantify losses in excitation intensity due to light-matter interaction (scattering, absorption, reflection) of the incident beam before reaching the target chromophores.

Is radiation damage the limiting factor in high-resolution single particle imaging with X-ray free-electron lasers?

Carl Caleman

Uppsala University, Sweden

carl.caleman@physics.uu.se

The prospect of single particle imaging with atomic resolution is one of the scientific drivers for the development of X-ray free-electron lasers. The assumption since the beginning has been that damage to the sample caused by intense X-ray pulses is one of the limiting factors for achieving subnanometer X-ray imaging of single particles and that X-ray pulses need to be as short as possible. Based on the molecular dynamics simulations of proteins in X-ray fields, we show that the noise in the diffracted signal caused by radiation damage is less than what can be expected from other sources, such as sample inhomogeneity and X-ray shot-to-shot variations. These findings show a different aspect of the feasibility of high-resolution single particle imaging using free-electron lasers, where employing X-ray pulses of longer durations could still provide a useful diffraction signal above the noise due to the Coulomb explosion.

X-ray Emission Spectroscopy at X-ray Free Electron Lasers: Limits to Observation of Unperturbed Electronic Structures

Yulia Pushkar,
Brendan Sullivan, Scott Jensen

Purdue University, Lafayette, Indiana, USA

ypushkar@purdue.edu

Modern free electron lasers provide intense X-ray pulses with ~1012 photons within ~10-100 femtoseconds. Such pulses enable new experimental techniques and provide unique opportunities for investigation of electronic and nuclear dynamics on their intrinsic time-scales. Interaction of ultra-bright, ultra-short X-ray pulses with matter can induce a multitude of nonlinear excitation processes which must be carefully considered when planning spectroscopic measurements and interpreting data recorded at XFELs. In most cases correct interpretation of the spectroscopic response and analysis of the electronic structure hinges on the assumption of single photon excitations. Here we attempted to answer the fundamental question on the limits to probing the ground (or native) electronic structure of a 3d transition metal ion at XEFL sources. Ions of the 3d transition metal (Mn2+) in a lighter element (O, C, H) environment were used as a model system. X-ray emission spectroscopy recorded from Mn2+ at different pulse conditions demonstrate spectral changes as a function of increased pulse intensity and pulse duration. To explain these changes, we develop a rate equation based on sequential ionization and relaxation events forming multiply ionized states during a single pulse which agree with observed spectroscopic trends. The percentage of Mn Kβ emission recorded after the 1st, 2nd and 3rd 1s ionization events is calculated from the developed rate equation model and validated by experimental measurements. A method for estimating shifts in atomic X-ray emission lines from sequential ionization during a single XFEL pulse is given. From our data we infer that, in addition to multiple ionization, the impact of electron cascades is more significant for longer pulses. We note that while use of shorter X-ray pulses will help to counteract additional effects of electron cascades it will not help to overcome the spectral shifts due to sequential ionization. Presented data and associated analysis will help with experimental designs at current and upcoming XFELs where even higher intensities and shorter pulses are expected. 3d elements have a variety of important applications such as in bio-inorganic catalysis, chemical catalysis and energy storage / conversion making robust protocols for their XFEL analysis of general importance.

Correcting Non-isomorphism

James M. Holton

University of California, San Francisco, USA

jmholton@lbl.gov

The problem of non-isomorphism has plagued macromolecular crystallography since the beginning [1-3], and it is essentially unavoidable in radiation damage studies. The unit cell changes with dose and that means the molecules in the cell must be adjusting somehow to the new cell. This will change the structure factors, but what if the molecular distortions could be corrected? Rigid-body motions cannot be the whole story because these lead to steric clashes. The true underlying distortion of the molecule must be both smooth across space, and also obey crystallographic symmetry. Here I present how periodic rubber-like distortions may be modelled using a collection of sine waves in space. This spatial distortion field (SDF) is similar in mathematical form to the Fourier synthesis of electron density from structure factors. The main differences are that the SDF is not a scalar field but a vector field describing changes in atomic position at every point in the unit cell. The number of orders needed to describe typical non-isomorphism is small, usually only 3-5 orders. SDFs may also be applied to electron density, allowing multi-crystal averaging across non-isomorphous crystal forms. Structural flexibility inherent to function may also be excited by these rubber-like distortions, making SDFs a potentially useful tool for elucidating subtle changes by eliminating the “noise” of rubber-like non-isomorphous distortion.

References

[1] B.S. Magdoff, F.H.C. Crick, Acta Cryst., 8 461 (1955).

[2] F.H.C. Crick, B.S. Magdoff, Acta Cryst., 9 901 (1956).

[3] C.C.F. Blake, R.H. Fenn, L.N. Johnson, D.F. Koenig, G.A. Mair, A.C.T. North, J.W.H. Oldham, D.C. Phillips, R.J. Poljak, V.R. Sarma, C.A. Vernon, in: M.G. Rossmann, E. Arnold (Eds.) International Tables for Crystallography F: Crystallography of biological macromolecules, Springer Netherlands, 2001. 745.

Dose calculations for microcrystallography, XFELs, and electron microscopy: extensions to RADDOSE-3D

Joshua Dickerson[1,2],
Elspeth Garman[2]

[1] MRC-LMB Cambridge, UK
[2] Department of Biochemistry, University of Oxford, UK

jdickerson@mrc-lmb.cam.ac.uk, elspeth.garman@bioch.ox.ac.uk

Radiation damage remains a fundamental limitation to the success of X-ray macromolecular crystallography (MX) experiments. The program RADDOSE-3D [1,2] estimates the dose absorbed by samples during data collection at synchrotron sources, allowing direct comparison of radiation damage between experiments carried out with different samples and beam parameters. Here, I present a number of extensions to RADDOSE-3D, which perform Monte Carlo simulations to improve the accuracy and applicability of RADDOSE-3D. The first of these extensions provides more accurate dose estimates for synchrotron data collection on microbeams or microcrystals by taking into account the redistribution of photoelectrons produced both in the crystal and the surrounding [3]. These emphasise the importance of beam energy [4,5,6], surrounding material, and crystal orientation on radiation damage to microcrystals. The second extension, RADDOSE-XFEL, calculates the time-resolved dose during XFEL data collection. The final extension, RADDOSE-EM, calculates the dose absorbed during single particle electron cryomicroscopy and micro-electron diffraction (MicroED) data collection. It is hoped that these extensions can be used to facilitate the study of radiation damage in new experiments and be used to maximise data collection efficiency.

References

[1] Zeldin, O. B., Gerstel, M. & Garman, E. F. RADDOSE-3D: Time- and space-resolved modelling of dose in macromolecular crystallography. J. Appl. Crystallogr. 46, 1225–1230 (2013).
[2] Bury, C. S., Brooks-Bartlett, J. C., Walsh, S. P. & Garman, E. F. Estimate your dose: RADDOSE-3D. Protein Sci. 27, 217–228 (2018).
[3] Dickerson JL & Garman EF. Absorbed doses in experiments with microbeams and microcrystals: Monte Carlo simulations in RADDOSE-3D. Protein Science. On-line July 2020
[4] Cowan, J. A. & Nave, C. The optimum conditions to collect X-ray data from very small samples. J. Synchrotron Radiat. 15, 458–462 (2008).
[5] Dickerson, J. L. & Garman, E. F. The potential benefits of using higher X-ray energies for macromolecular crystallography. J. Synchrotron Radiat.
[6] Storm, S. L. S. et al. Measuring energy-dependent photoelectron escape in microcrystals. IUCrJ 7, 129–135 (2020)

Quantitative simulation tools for predicting radiation damage driven by high-intensity x-ray pulses

Robin Santra

DESY & Universität Hamburg, Hamburg, Germany

robin.santra@cfel.de

One of the key opportunities offered by the development of x-ray free-electron lasers is the determination, at atomic resolution, of the three-dimensional structure of biologically relevant macromolecules. The basic idea underlying molecular imaging using x-ray free-electron lasers is the ``diffract-and-destroy'' concept: If one uses an x-ray pulse that is sufficiently short (on the order of femtoseconds), then in a single shot an x-ray scattering pattern may be obtained that is practically unaffected by atomic displacements triggered by ionization events during the x-ray pulse. What cannot be eliminated in this way is the impact of the electronic damage on the x-ray scattering patterns. Theory, therefore, plays an important role in the development of this new imaging technique: A quantitative understanding is required of the damage processes occurring during the exposure of a molecule to an ultraintense, ultrafast x-ray pulse. In this talk, I will present progress we have made in order to address this challenge. One tool we have developed, XMDYN, is a molecular-dynamics code that utilizes ab-initio atomic electronic-structure information, computed on the fly, within a Monte-Carlo framework. XMDYN has been successfully tested through experiments at LCLS and SACLA. XMDYN is part of a powerful start-to-end simulation framework for single-particle imaging at the European XFEL. Recently, we have taken first steps towards a full ab-initio framework for simulating high-intensity x-ray–matter interactions. Our new XMOLECULE software solves the polyatomic quantum-mechanical electronic-structure problem for every electronic state arising during the exposure of a molecule to a strong x-ray pulse. From this information, electronic transition rates (such as Auger decay rates) are computed on the fly, and the associated rate equations are integrated utilizing a Monte-Carlo method. XMOLECULE played a key role in a recent LCLS experiment on iodomethane, in which hard x-rays focused to a peak intensity exceeding 1019 W/cm2 produced the highest charge states ever formed using light. Not only did XMOLECULE correctly predict the charge-state distribution observed, but it also helped identify a new molecular ionization enhancement mechanism based on intramolecular charge transfer.

Radiation damage in X-ray spectroscopy

Pieter Glatzel

ESRF, Grenoble, France

glatzel@esrf.fr

X-ray spectroscopy probes the electronic structure around an analyte element in a sample. The electronic structure relates to the formal oxidation and spin state and the atomic structure around the analyte element. The sensitivity to small modification of the electronic structure makes X-ray spectroscopy strongly responsive to small changes of the sample due to X-ray irradiation. Thus, X-ray spectroscopy often tolerates lower doses than atomic structural probes such as crystallography. The effect of X-rays in the sample may be oxidation or reduction of a metal site accompanied by modification of the local atomic coordination. Often, X-rays induce changes similar to other external triggers such as optical/UV illumination or temperature. This can be used to understand the changes in the sample, i.e. X-rays serve as source and probe of the sample modification. Radiation damage dramatically changes the experimental protocol to record spectroscopic data and some measurements become impossible. The presentation provides examples and discusses some fundamental concepts.

An Objective Metric for Correcting Radiation Damage in SAXS

Yunyun Gao[1],
Timothy Stachowski[2], Thomas Grant[2], Arwen Pearson[3], Edward H. Snell[2]

[1] Universität Hamburg/ Max-Planck Institute for Structure and Dynamics of Matter, Hamburg, Germany
[2] Hauptmann Woodward Institute, USA
[3] Astbury Centre for Structural Molecular Biology, University of Leeds, UK

yunyun.gao@cfel.de

Radiation damage remains a fundamental limitation to the success of X-ray macromolecular crystallography (MX) experiments. The program RADDOSE-3D [1,2] estimates the dose absorbed by samples during data collection at synchrotron sources, allowing direct comparison of radiation damage between experiments carried out with different samples and beam parameters. Here, I present a number of extensions to RADDOSE-3D, which perform Monte Carlo simulations to improve the accuracy and applicability of RADDOSE-3D. The first of these extensions provides more accurate dose estimates for synchrotron data collection on microbeams or microcrystals by taking into account the redistribution of photoelectrons produced both in the crystal and the surrounding. These emphasise the importance of beam energy [3,4,5], surrounding material, and crystal orientation on radiation damage to microcrystals. The second extension, RADDOSE-XFEL, calculates the time-resolved dose during XFEL data collection. The final extension, RADDOSE-EM, calculates the dose absorbed during single particle electron cryomicroscopy and micro-electron diffraction (MicroED) data collection. It is hoped that these extensions can be used to facilitate the study of radiation damage in new experiments and be used to maximise data collection efficiency.

References

[1] Blanchet, C.E., Spilotros, A., Schwemmer, F., et al. 2015. Versatile sample environments and automation for biological solution X-ray scattering experiments at the P12 beamline (PETRA III, DESY). Journal of Applied Crystallography 48(2), pp. 431–443.
[2] Brennich, M.E., Kieffer, J., Bonamis, G., et al. 2016. Online data analysis at the ESRF bioSAXS beamline, BM29. Journal of Applied Crystallography 49(1), pp. 203–212.
[3] Franke, D., Jeffries, C.M. and Svergun, D.I. 2015. Correlation Map, a goodness-of-fit test for one-dimensional X-ray scattering spectra. Nature Methods 12(5), pp. 419–422.
[4] Rambo, R.P. and Tainer, J.A. 2013. Accurate assessment of mass, models and resolution by small-angle scattering. Nature 496(7446), pp. 477–481.
[5] Ryan, T.M., Trewhella, J., Murphy, J.M., et al. 2018. An optimized SEC-SAXS system enabling high X-ray dose for rapid SAXS assessment with correlated UV measurements for biomolecular structure analysis. Journal of Applied Crystallography 51(1), pp. 97–111.

An Objective Metric for Correcting Radiation Damage in SAXS

Maciej Kozak

Department of Macromolecular Physics, Adam Mickiewicz University, Poznań, Poland

ma.kozak@uj.edu.pl

During small angle X-ray scattering studies of proteins in solutions, radiation damages may occur during exposure to the synchrotron radiation, which most often involves the protein aggregation process, but global conformational changes such as the domain swapping phenomenon is also possible.

The domain swapping phenomenon has been known for several decades and has been identified in numerous protein structures [1]. Particularly was observed for proteins of flexible structure. The phenomenon of domain swapping also accompanies of the amyloidogenesis process. This situation applies also to human cystatin C, where dimerization and oligomerization of this protein occurs through domain swapping [2,3].

In our previous studies, we observed domain swapping in the crystal structure of human cystatin C, where it probably occurred during the crystallization process. Recently we also observed, that this phenomenon for human cystatin C also occurs in solution and is caused by irradiation during SAXS experiments using synchrotron radiation [4]. During the lecture it will be discussed the radiation induced domain swapping of human cystatin C and other proteins.

References

[1] Liu, Y., Eisenberg, D. Protein Sci. 11(6), 1285-1299 (2002).
[2] Janowski, R. Kozak, M., Jankowska, E. Grzonka, Z. Abrahamson, M., Grubb, A. & Jaskolski, M. Nature Struct. Mol. Biol. 8, 316–320 (2001).
[3] Janowski, R., Kozak, M., Abrahamson, M., Grubb, A. & Jaskolski, M. Proteins: Struct. Funct., Bioinf. 61, 570–578 (2005).
[4] Taube, M., Pietralik, Z., Szymanska, A., Szutkowski, K., Clemens, D., Grubb, A., Kozak, M. Sci Rep 9, 8548 (2019).

Posters

You have the chance to ask questions and discuss their research with the presenters, using the ZOOM links provided on our webpage, during the following times:

Session A: During "*Tea and Posters 1*", Wednesday, 14th, 15:20-15:40 CEST

Session B: During "*Tea and Posters 2*", Thursday, 15th, 15:50-16:10 CEST

Session C: During "*Tea and Posters 3*", Friday, 16th, 15:20-15:40 CEST

Poster	Title
A1	**X-ray radiation induced TGFβ-1 activation: setting the record straight using solution X-ray scattering** Timothy R. Stachowski
A2	**The MANACA beamline at Sirius, structural biology at 4th generation** Andrey Fabricio Ziem Nascimento
A3	**Functional dynamics of a single tryptophan residue in a BLUF protein revealed by fluorescence spectroscopy** Sofia M. Kapetanaki
A4	**Crystal defects in high-quality protein crystals induced by X-ray irradiation** Ryo Suzuki
A5	**Water radiolysis quantification upon soft X-rays exposure using a microfluidic cell** Lucie Huart
B1	**RETRACTED**
B2	**Studying disulfide damage at low X-ray doses with an engineered protein approach** Edward Snell
B3	**RETRACTED**
B4	**RETRACTED**
B5	**Solution-based high-energy SAXS measurements on metallic nanoparticles and photo-switchable lipid vesicles** Martina Ober, Bert Nickel
C1	**Valence Photoionization of Thymine: Threshold Photoelectron Spectrum and Dissociative Photoionization studied with Photoelectron Photoion Coincidence (PEPICO) Spectroscopy** Andras Bodi
C2	**Fixed target FEL crystallography for Phasing at 7.2 KeV** Rajendran Chitra
C3	**Towards systematic dosimetry on SOLEIL's Proxima 2A beamline** Martin Savko
C4	**Data Collection Strategies for the Radiation Sensitive, Ferric Iron Binding Protein, FutA.** Rachel Bolton