

3. - 5. June 2025  
Paul Scherrer Institute, Switzerland

12<sup>th</sup> International Workshop  
on Radiation Damage to  
Biological Samples



# Book of Abstracts

The PSI logo consists of a circular arrangement of small, shimmering white particles that form a larger, glowing shape.

PSI

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## General Information

### Getting to PSI

The workshop will take place in the Auditorium (WHGA) in the PSI WEST campus.

If you stay at the Centurion Hotel, please take Bus 376 from BRUGG to PSI WEST. The Auditorium is the red building just opposite the bus stop PSI WEST.

### Access to PSI

The workshop takes place in the publicly accessible part of PSI. No additional registration is needed.

However, if you need access to the PSI Campus, please arrange with on-site staff at least 24h before to make sure we can get you registered for badge and dosimeter.

### Registration Desk

The registration desk will be located just outside the Auditorium.

### Wi-Fi

Information about the on-site Wi-Fi and the weekly password will be provided at the beginning of the workshop.

### Coffee Breaks

Complimentary drinks and snacks during coffee breaks will be served outside the Auditorium next to the poster boards.

### Bathrooms

Bathroom facilities can be found in the basement below the Auditorium.

### Workshop Dinner

The Workshop dinner will take place at FHNW Windisch Campus, right next to Hotel Centurion.

There will be a bus transfer from PSI to FHNW, leaving from the PSI WEST bus stop. Please make sure you are at the bus stop no later than **18:50** on day 2, as the bus will leave 19:00 sharp.

## Tour SLS/SwissFEL

On your name tag you can find a letter (A-D) corresponding to your tour group. If you intend to join the tour, please go to the tour guide holding up your letter outside the Auditorium at **14:25** on day 2

The tour involves approx. 445min of walking between SLS and SwissFEL. If you need assistance. Please let the staff know.

## Check-in to Hotel centurion after 22:00

Please be aware that the reception at Centurion closes at 22:00. If you arrive later, please use the code **2505** at the door. Then you will find an envelope with your name, containing your room key, on the reception desk. Please go to the reception in the morning to complete the check-in.

In case of emergency, please call +41 056 460 22 22

## Busses back to Hotel

There will be no organized transport back to Brugg on the first day, to allow everybody to stay as long or short as they want for our aperó.

Please use the Bus 376 to Brugg, leaving at 19:31, 19:36, 20:01, 20:06, 20:36, 21:01, 21:06, or 22:06.

## Emergencies

In case of emergencies, please contact our staff or dial 3333 from a PSI phone or +41 56 310 3333 from a mobile phone.

## Presenter Information

### Speaker Instructions

We ask that all presenters to come and check that their laptop and presentation works with the AV equipment in the break before their respective session.

If you don't bring your own laptop for the presentation, please let us know and we can copy your PowerPoint slides from USB to our presentation device.

All presentations have a 20 minute format, plus 5 more minutes for questions. Please be aware that we ask all chairs to keep strict times as we have a tight schedule.

### Poster Instructions

Please find your poster number in the table below.

Poster boards will be available in the lobby from the auditorium. Please hang your poster up on the corresponding board at the beginning of the workshop and leave them up till the end. Posters left after the end of the workshop will be discarded.

The size of the poster boards will be DIN A0. The panels will have a portrait orientation. There will be pins available for mounting your posters.

**Tuesday 3 June**

12:00	<p><b>Catering: Registrarion &amp; Welcome Lunch</b>                  Session   Location: Paul Scherrer Institut, Auditorium Entrance</p>
13:00	<p><b>General: Welcome and Introduction</b>                  Session   Location: Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland                    Convener: Elspeth GARMAN</p>
13:15	<p><b>Biological Studies Affected by Radiation Damage: BIO 1</b>                  Session   Location: Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland                    Convener: Kunio HIRATA</p>
13:15	<p>13:15-13:45  <b>Rejuvenating online UV-Vis microspectrophotometry by monitoring dose- and time-resolved phenomena at both cryogenic and room temperature</b>                  Speaker                  Sylvain ENGLIBERGE</p>
	<p>13:45-14:15  <b>Specific Radiation Damage to Halogenated Inhibitors and Ligands in Protein-Ligand Crystal Structures</b>                  Speaker                  Matthew J. RODRIGUES</p>
	<p>14:15-14:45  <b>Capturing X-ray-Induced Photo-Reduction in Arsenite Oxidase: Implications for the Catalytic Mechanism</b>                  Speaker                  Filipa ENGROLA</p>
	<p>14:45-15:15  <b>Diffraction Intensity as a Radiation Damage Progression Metric and Intensity Decay Models</b>                  Speaker                  Elspeth GARMAN</p>
15:15	<p><b>Catering: Coffee</b>                  Session   Location: Paul Scherrer Institut, Auditorium Entrance</p>
15:45	<p><b>Damage at New Sources - XFELs and 4th Generation Synchrotrons</b>                  Session   Location: Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland                    Convener: Valérie PANNEELS</p>
15:45	<p>15:45-16:15  <b>A protein switch to bind different redox states in the cyanobacterial FutA iron binding protein revealed by an X-ray pump-probe approach</b>                  Speaker                  Ivo TEWS</p>

17:45	<p><b>16:15–16:45</b>    <b>Radiation Damage Aspects in Ultra-High-Resolution Single-Crystal MX</b></p> <p><b>Speaker</b> Gleb BOURENKOV</p>
17:45	<p><b>16:45–17:15</b></p> <p><b>Investigation of radiation damage in room temperature serial macromolecular crystallography at a fourth generation Synchrotron</b></p> <p><b>Speaker</b> Samuel L. ROSE</p>
18:15	<p><b>17:15–17:45</b></p> <p><b>Damage before destruction? X-ray-induced changes in single-pulse serial femtosecond crystallography</b></p> <p><b>Speaker</b> Robin OWEN</p>
18:15	<p><b>17:45</b></p> <p><b>Posters: Poster Blitz</b></p> <p><b>Poster Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland   <b>Convener:</b> Florian DWORKOWSKI</p>
19:00	<p><b>18:15</b></p> <p><b>Discussion</b></p> <p><b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland   <b>Conveners:</b> Nadia ZATSEPIN, Michael HOUGH</p>
20:00	<p><b>19:00</b></p> <p><b>Catering: Dinner</b></p> <p><b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium Entrance</p>
21:30	<p><b>20:00</b></p> <p><b>Posters: Posters &amp; Drinks</b></p> <p><b>Poster Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland</p>

## Wednesday 4 June

09:00	<p><b>Biological Studies Affected by Radiation Damage: BIO 2</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland    <b>Convener:</b> Edward SNELL</p> <p><b>09:00-09:30</b>  <b>X-ray photoreduction of the active site copper in the fungal lytic polysaccharide monoxygenase LsAA9A</b>  <b>Speaker</b>          Leila LO LEGGIO</p> <p><b>09:30-10:00</b>  <b>Breaking Bad...or not...: altering experimental conditions to probe bond fate</b>  <b>Speaker</b>          Roberto STEINER</p>
10:00	<p><b>Radiation Damage in Electron Crystallography and Microscopy</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland    <b>Convener:</b> Thomas URSBY</p> <p><b>10:00-10:30</b> <b>TEM and STEM Imaging of Radiation-Sensitive Samples</b>  <b>Speaker</b>          Ray EGERTON</p> <p><b>10:30-11:00</b> <b>Extending the reach of single-particle cryoEM</b>  <b>Speaker</b>          Christopher J. RUSSO</p>
11:00	<p><b>Catering</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium Entrance</p>
11:30	<p><b>Radiation Damage in Electron Crystallography and Microscopy</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland    <b>Convener:</b> Gerhard HOFER</p> <p><b>11:30-12:00</b> <b>Perspectives and limitations in high-resolution cryo-EM</b>  <b>Speaker</b>          Holger STARK</p> <p><b>12:00-12:30</b> <b>A Physical Theory For Cryo-EM at Liquid-Helium Temperatures</b>  <b>Speaker</b>          Joshua DICKERSON</p> <p><b>12:30-13:00</b>  <b>Cryo-EM structure of photosystem II reveals damages caused by commonly used electron doses in imaging</b>  <b>Speaker</b>          Koji KATO</p>

13:30	<p><b>13:00-13:30 Radiation damage in recent MicroED measurements</b></p> <p><b>Speaker</b> Johan HATTNE</p>
13:30	<p><b>Catering: Lunch</b></p> <p><b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium Entrance</p>
14:30	<p><b>General: Visit SLS2.0 and SwissFEL</b></p> <p><b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland</p>
16:00	<p><b>Catering: Coffee</b></p> <p><b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium Entrance</p>
16:30	<p><b>Radiation Damage in Complementary Fields including Biological Imaging</b></p> <p><b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland   <b>Convener:</b> Daniele DE SANCTIS</p>
16:30	<p><b>16:30-17:00</b></p> <p><b>High-dose effects in high-resolution X-ray microscopy of soft materials</b></p> <p><b>Speaker</b> Ana DIAZ</p>
17:00	<p><b>17:00-17:30</b></p> <p><b>Coherent X-rays Reveals Radiation-Induced Dynamics in Hydrated Proteins</b></p> <p><b>Speaker</b> Foivos PERAKIS</p>
17:30	<p><b>17:30-18:00</b></p> <p><b>Radiation damage in time-resolved X-ray solution scattering experiments</b></p> <p><b>Speaker</b> Magnus P. ANDERSSON</p>
18:00	<p><b>Discussion</b></p> <p><b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland   <b>Conveners:</b> James HOLTON, Briony YORKE</p>
18:45	<p><b>General: Transfer to Brugg</b></p> <p><b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland</p>
19:30	<p><b>Catering: Conference Dinner</b></p> <p><b>Session</b>   <b>Location:</b> FHNW Windisch</p>
22:30	

## Thursday 5 June

09:00	<p><b>Pump-Laser Excitation Conditions in Time-Resolved Serial Femtosecond Crystallography</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland    <b>Convener:</b> Philip JOHNSON</p>
	<p>09:00-09:30  <b>Ultrafast structural changes in myoglobin: influence of pump laser fluence</b>  <b>Speaker</b>  Thomas R.M. BARENDs</p>
	<p>09:30-10:00  <b>Time-resolved serial femtosecond crystallography on the fluorescent protein rsEGFP2-V151A in different photon excitation regimes</b>  <b>Speaker</b>  Nicolas COQUELLE</p>
	<p>10:00-10:30  <b>Structural effects of high laser power densities on an early bacteriorhodopsin photocycle intermediate</b>  <b>Speaker</b>  Jörg STANDFUSS</p>
10:30	
10:30	
11:00	<p><b>Catering: Coffee</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium Entrance</p>
11:00	<p><b>Radiation Damage in Temperature Controlled Crystallography</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland    <b>Convener:</b> Vincent OLIERIC</p>
	<p>11:00-11:30  <b>Mapping the Conformational Landscapes of Human Kinases with Multi-Temperature X-ray Crystallography</b>  <b>Speaker</b>  Michael THOMPSON</p>
	<p>11:30-12:00  <b>Exploring Ligand-Protein Interactions at Multiple Temperatures Using Macromolecular Crystallography</b>  <b>Speaker</b>  Chia-Ying HUANG</p>
12:00	
12:00	<p><b>Discussion</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland    <b>Conveners:</b> Arwen PEARSON, Colin NAVE</p>
12:45	
12:45	<p><b>General: Summary and Farewell</b>  <b>Session</b>   <b>Location:</b> Paul Scherrer Institut, Auditorium, Forschungsstrass 111 5232 Villigen PSI Switzerland    <b>Convener:</b> Martin WEIK</p>
13:00	

# Talks

Day 1

## *Session 1: Biological Studies Affected by Radiation Damage 1*

Tuesday, June 3<sup>rd</sup> | 13:15

### Rejuvenating online UV-Vis microspectrophotometry by monitoring dose- and time-resolved phenomena at both cryogenic and room temperature

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*Sylvain Engilberge*  
*IBS Grenoble*

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The French protein crystallography beamline BM07-FIP2 at the ESRF enables both cryogenic and room-temperature studies on single crystals, with precise control over the deposited X-ray dose thanks to a large, homogeneous top-hat beam. [1] In addition, its sample environment allows for easy integration of the EMBL/ESRF microspectrophotometer [2], enabling in crystallo UV-Visible absorption and fluorescence measurements in parallel with X-ray diffraction. This approach has allowed for the monitoring of the evolution of the absorbance of metal centres, cofactors, or chromophores as a function of X-ray dose, providing real-time insights into both the extent of radiation damage and the functional state of macromolecules. This presentation will focus on recent methodological developments that led studying the extent of radiation damage on various proteins, enabling the comparison between room and cryogenic temperature. Finally, the plans for the future sample environment of BM07-FIP2 fully integrating an improved microspectrophotometer will be described.

#### *References*

[1] McCarthy A. et al. (2025) *J Synchrotron Radiat.*, 32, in press.

[2] McGeehan J, Ravelli RB, Murray JW, Owen RL, Cipriani F, McSweeney S, Weik M, Garman EF. (2009) *J Synchrotron Radiat.*, 16, 163-172.

## Specific Radiation Damage to Halogenated Inhibitors and Ligands in Protein-Ligand Crystal Structures

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*Matthew Rodrigues*  
*Diamond Light Source*

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Protein–inhibitor crystal structures aid medicinal chemists in efficiently improving the potency and selectivity of small-molecule inhibitors. It is estimated that a quarter of lead molecules in drug discovery projects are halogenated. Protein–inhibitor crystal structures have shed light on the role of halogen atoms in ligand binding. They form halogen bonds with protein atoms and improve shape complementarity of inhibitors with protein binding sites. However, specific radiation damage (SRD) can cause cleavage of carbon–halogen (C–X) bonds during X-ray diffraction data collection. This study shows significant C–X bond cleavage in protein–ligand structures of the therapeutic cancer targets B-cell lymphoma 6 (BCL6) and heat shock protein 72 (HSP72) complexed with halogenated ligands, which is dependent on the type of halogen and chemical structure of the ligand. The study found that metrics used to evaluate the fit of the ligand to the electron density deteriorated with increasing X-ray dose, and that SRD eliminated the anomalous signal from brominated ligands. A point of diminishing returns is identified, where collecting highly redundant data reduces the anomalous signal that may be used to identify binding sites of low-affinity ligands or for experimental phasing. Straightforward steps are proposed to mitigate the effects of C–X bond cleavage on structures of proteins bound to halogenated ligands and to improve the success of anomalous scattering experiments.

### *References*

[1] Rodrigues, M.J., Cabry, C., Collie, G., Carter, M., McAndrew C., Owen, R.L., Bellenie, B.R., Le Bihan, Y-V., van Montfort, R.L.M. (2024) J. Appl. Cryst., 57, 1951-1965.

## Capturing X-ray-Induced Photo-Reduction in Arsenite Oxidase: Implications for the Catalytic Mechanism

Filipa Engrola  
UCIBIO

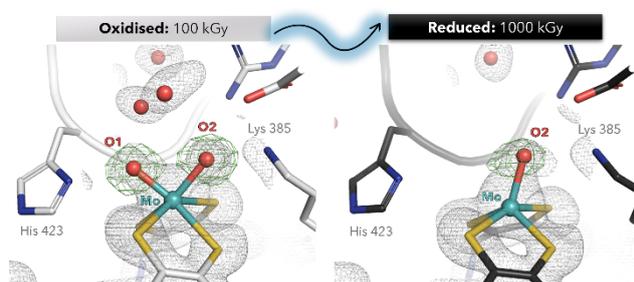
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The molybdenum enzyme arsenite oxidase (Aio), is a promising biocatalyst for the detoxification and biosensor applications [1]. So far, its structural characterization and complete reaction mechanism understanding has been limited to artifactually reduced states of the Mo cofactor (Moco) caused by X-ray photoreduction (PDB 1g8k, 4aay) [2-4].

Here, we present the first crystallographic evidence of X-ray-induced photo-reduction in *Alcaligenes faecalis* Aio, tracking active-site geometric changes across incremental radiation doses (100–1000 kGy) at the PETRA III P14 beamline. High-resolution structures (1.5 Å) reveal that photo-reduction triggers the loss of a labile oxo ligand (O1), while displacing the Mo atom toward the dithiolene plane.

Low-dose data (100 kGy) allowed us to capture, for the first time, the Mo(VI) centre in a six-coordinated geometry with asymmetric cis-dioxo coordination (Mo–O1: 1.8 Å; Mo–O2: 2.1 Å). Reduction of the Moco also flattened the twist angle of the pterin from 31° to 17°, modulating the active site catalysis.

Our work demonstrates that radiation damage artifacts—prevalent in metalloenzyme crystallography—can obscure mechanistic insights. These findings underscore the need for dose-optimized structural data to refine AI-driven models of metalloenzyme mechanisms and advance the rational engineering of Aio for biosensing and bioremediation.



### References

- [1] Male, K.B. et al. (2007) *Anal. Chem.*, 79(20), 7831–7837.
- [2] Warelw T.P. et al., (2013) *PLoS One*, 8(8): e72535. [3] Ellis, P.J. et al. (2001) *Structure*, 9(2), 125-132.
- [4] Engrola, F. et al. (2023) *J. Biol. Chem.*, 299(8), 105036.

## Diffraction Intensity as a Radiation Damage Progression Metric and Intensity Decay Models

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*Elspeth Garman*  
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Ever since the first systematic study of radiation damage in macromolecular crystallography, diffraction intensity decay has been useful as one of the metrics to monitor the progression of radiation damage in reciprocal space for room temperature [1] and cryo-cooled (~100 K) [2] crystalline samples. Various models have been used to parameterise the functional form of the intensity decay (IDMs), but none thus far have been completely satisfactory in both fitting the data and having physically interpretable parameters. The absorbed dose is the most useful x-axis against which to plot intensity decay, since it allows different experimental arrangements to be compared, and the program RADDOSSE-3D [3,4] was developed to allow convenient dose estimation.

Results from the recent inclusion of the option to enter an IDM [5] into RADDOSSE-3D will be presented. The IDM enables the previous RADDOSSE-3D output of 'Diffraction Weighted Dose' to be modified from a 'Fluence Weighted Dose' to a 'Diffraction-Decay Weighted Dose' [6], allowing more informed decisions to be made on possible data collection strategies. Over the last 10 years RADDOSSE-3D [4] has been extended for use in a wide variety of diffraction and scattering experiments (MX, SMX, XFEL, SAXS, XPS, PXRD) and most recently, electron diffraction, RADDOSSE-ED [6]. In addition, we have now released a new RADDOSSE-3D GUI [6,7] which allows the estimation of dose for any of these modalities.

### References

- [1] Blake, C.C.F., Phillips, D.C. In Proceedings of the Symposium on the Biological Effects of Ionising Radiation at the Molecular Level (Vienna: International Atomic Energy Agency), (1962) pp. 183–191
- [2] Gonzales, A., Nave, C. Acta Cryst. D (1994) 50, 874–877
- [3] Zeldin, O.B., Gerstel, M., Garman, E.F. J. Appl. Cryst (2013) 46, 1225–1230
- [4] Bury, C.S., Brooks-Bartlett, J.C., Walsh, S.P., Garman, E.F. Protein Science (2018) 27, 217–228
- [5] Leal, R.M., Bourenkov, G., Russi, S., Popov, A.N. J Synchrotron Radiat. (2013) 20(1), 14–22
- [6] Dickerson, J.L., McCubbin, P.T.N., Brooks-Bartlett, J.C., Garman, E.F. Protein Science (2024) 33:e5005.  
<https://github.com/GarmanGroup/RADDOSSE-3D>

Tuesday, June 3<sup>rd</sup> | 15:45

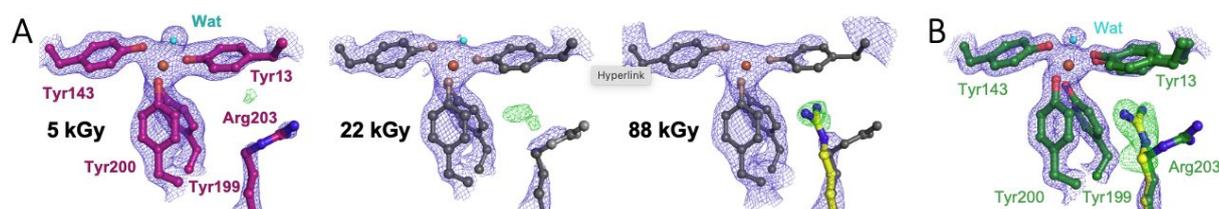
## A protein switch to bind different redox states in the cyanobacterial FutA iron binding protein revealed by an X-ray pump-probe approach

Ivo Tews  
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Radiation damage is a faithful attender to X-ray crystallographic studies of metallo-proteins. Thus, care is taken to limit the effects of radiation damage and avoid consequent misinterpretation of X-ray crystallographic results. In our study, we applied defined doses to selectively probe the redox states involved in metal binding.

We studied the cyanobacterial iron binding protein FutA, an ABC transporter substrate binding protein that can also act as an intracellular iron binding protein [1]. We determined crystallographic structures using X-ray and neutron radiation characterised as ferrous [Fe(II)] and ferric [Fe(III)] complexes [2]. These states are distinguished by protein conformation, particularly the positioning of the positively charged Arg203 side chain as part of the iron binding site in the [Fe(II)] complex.



We captured the transition between [Fe(III)] and [Fe(II)] states upon X-ray photoreduction with a dose series using a serial synchrotron crystallography fixed target approach, see panel A. Using a novel XFEL X-ray pump-probe approach, we uncovered how Arg203 functions as a molecular switch, enabling accommodation of different iron oxidation states, see panel B [2]. The switching capability of the single FutA protein provides functional insight and suggests genome streamlining, where the loss of specialised FutA variants may reflect ecological adaptation.

### References

- [1] Polyviou, D. et al. (2018) *J. Biol. Chem.*, 293, 18099-18109.
- [2] Bolton, R. et al. (2024) *PNAS*, 121, e2308478121.

## Radiation Damage Aspects in Ultra-High-Resolution Single-Crystal MX

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*Gleb Bourenkov*  
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solely deciphering structures and refining atomic positions, to the analysis of subtle structural effects. Examples involve unpredicted deviations in molecular geometries; the details of protonation states of functional groups, ligands and solvent molecules; occupancies of mixed states; atomic vibrations and, finally, electron density studies of active sites and ligands. Classical high-resolution cryogenic single-crystal MX remains an essential complement to time-resolved methods. The underlying data collection methodologies, in which radiation damage continues to be the most important consideration, are evolving from being able to predict diffraction half-life times towards providing optimal choices both delivering the highest data precision and preserving the interpretability of subtle structural features. In practice, virtually every such feature must be experimentally tested to exclude radiation damage artifacts.

The use of large, homogeneous beams at high photon energies up to 30 keV, in combination with high-Z detectors and advanced multi orientation data collection strategies [1, 2] are key. Given that ultra-high resolution diffraction experiments rely on the use of large, coherently diffracting crystals these experiments necessarily require large, top-hat X-ray beams. This is also a prerequisite for precise and reproducible X-ray dosage. We will review the instrumental aspects involved in creating and using such beams at modern beamlines, as well as the possibilities offered by the potential of fourth-generation synchrotron sources.

Finally, we will present the analysis of dose series collected on several ultra-high-resolution structures, both in reciprocal and in real space, in conjunction with previously proposed radiation-damage models [3, 4, 5].

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## Investigation of radiation damage in room temperature serial macro-molecular crystallography at a fourth generation Synchrotron

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Radiation damage is a major concern in macromolecular crystallography (MX) where ionising X-rays used for structure determination can result in a cascade of damaging processes caused by the absorption of energy (denoted mainly as dose: absorbed energy/mass (J/kg; Gray (G)) and radiolysis of molecules. These can introduce artefacts within the structure (specific damage) and the overall data (global damage). Collection at cryogenic temperatures (100 K) is normally performed to mitigate the effects of radiation damage but recently there has been a resurgence in room temperature (RT) and serial crystallography collection. Serial synchrotron crystallography (SSX), with approaches adopted from X-ray free electron lasers (XFELs), provides a suitable way to collect RT data at synchrotrons with reduced radiation damage by spreading the total dose over thousands of individual microcrystals. This usually relies on a microfocus beamline and a very low-dose per crystal collection strategy to compensate.

With advancements in technologies, synchrotrons are now being upgraded or constructed to fourth generation light sources, offering much higher brilliance compared to their predecessors, and, consequently, microfocus MX beamlines with increased flux density are becoming more routinely available offering much higher dose rates for RT-SSX collection. One example of this is ID29, the flagship beamline constructed at the high-energy fourth generation European Synchrotron Radiation Facility (ESRF), following its upgrade to the Extremely Brilliant Source (ESRF-EBS) [Raimondi et al, 2023]. ID29 is currently in unique territory, delivering slightly polychromatic (1%  $\Delta E/E$ ) microsecond X-ray pulses (90  $\mu$ s) with a flux density of  $> 10^{14}$  ph/s/ $\mu$ m<sup>2</sup>, three times higher than third generation sources, and with dose rates on the order of several GGy/s. The unique beam characteristics on ID29 allow for the possibility of serial microsecond crystallography (S $\mu$ X) for the structure determination of macromolecules using RT-SSX [Orlans et al, 2025]. As ID29 is setting a precedent for similar beamlines that are appearing or due to appear worldwide, a comprehensive radiation damage analysis is thus required.

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Tuesday, June 3<sup>rd</sup> | 17:15

## Damage before destruction? X-ray-induced changes in single-pulse serial femtosecond crystallography

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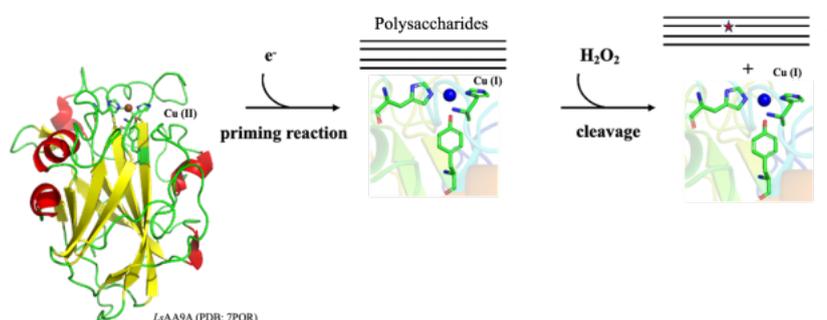
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Serial femtosecond crystallography (SFX) exploits extremely brief X-ray free-electron laser pulses to obtain diffraction data before destruction of the crystal. However, during the pulse X-ray-induced site-specific radiation damage can occur, leading to electronic state and/or structural changes. Here, we present a systematic exploration of the effect of single-pulse duration and energy (and consequently different dose rates) on site-specific radiation damage under typical SFX room-temperature experimental conditions. For the first time in SFX we directly measured the photon pulse duration, varying from less than 10 fs to more than 50 fs, and used three pulse energies to probe in-pulse damage in two radiation-sensitive proteins: the iron-heme peroxidase DtpAa and the disulfide-rich thaumatin. While difference-map features arising from radiation damage are observed, they do not lead to significant change in refined atomic coordinates or key bond lengths. Our work thus provides experimental verification that average atomic coordinates are not significantly perturbed by radiation damage in typical SFX experiments.

## X-ray photoreduction of the active site copper in the fungal lytic polysaccharide monooxygenase LsAA9A

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Lytic polysaccharide monooxygenases are enzymes [1] binding their active-site copper through the characteristic His-brace motif shown above including two His – one N-terminal – and often also a Tyr. The reaction cycle starts with reduction of the resting state Cu(II) to Cu(I) – in the laboratory usually using ascorbate as small molecule reductant. Despite the name, most LPMOs prefer hydrogen peroxide as co-substrate, to subsequently oxidatively cleave the glycosidic bonds in saccharides.

We have previously – through crystal cryo-structures of the model enzyme LsAA9A determined at high and low X-ray doses, and based on the hypothesis that X-ray induced photoreduction mimics natural priming reaction - reconstructed possible changes in geometry during the catalytic cycle and identified a small shortening of the Cu(II)-Tyr distance [2].

Aside from uncertainty on the biological significance of such shortening [3], we wanted to address concerns regarding the ability of macromolecular crystallography to reliably detect differences in the order of 0.1-0.2 Å. We thus carried out additional triplicate independent structure determination representing Cu(I)/Cu(II) states with/without the model substrates celotriose, showing statistically significant differences only for the Cu(II)-Tyr distance with/without saccharide, but no other Cu-protein distance.

In order to assess whether additional general X-ray damage obscures similar shortening in the Cu(I) state induced by photoreduction, we are now comparing with cryo data collected after priming by chemical reduction with ascorbate at low X-ray doses.

Finally since X-ray-induced photoreduction of the active-site copper may closely approximate the chemical priming reaction, it holds potential as a trigger for time-resolved studies. To explore this possibility further, we are currently investigating the photoreduction process at room temperature.

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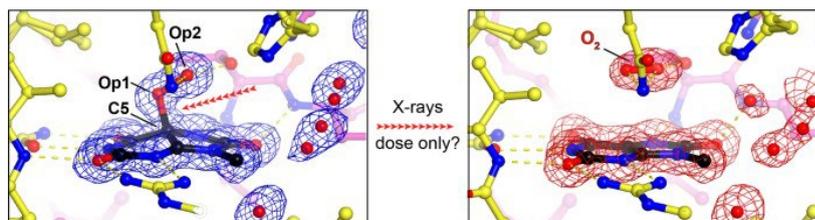
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## Breaking Bad...or not...: altering experimental conditions to probe bond fate

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Urate oxidase (UOX) is the archetypal cofactor-independent oxidase, catalysing the O<sub>2</sub>-mediated degradation of uric acid in many organisms. Previously, we demonstrated that the first step of this reaction is a dioxygenation reaction, forming 5-peroxyisourate (5-PIU) as an intermediate [1]. We also showed that 5-PIU is highly sensitive to radiation: during conventional single-axis X-ray diffraction experiments at 100 K, doses between 50-100 kGy caused visible damage to the C5–Op1 bond, and doses around 200 kGy led to complete bond rupture, accompanied by in situ release of O<sub>2</sub> [1,2]. Surprisingly, a subsequent synchrotron serial crystallography (SSX) experiment at room temperature (RT) showed no damage to this bond, even at an estimated dose of up to 70 kGy [3]. In this talk, I will discuss our more recent experiments exploring radiation-induced bond rupture under various experimental conditions—including gaussian vs. top-hat beam profiles, cryogenic vs. room temperature, and serial vs. conventional crystallography—in an effort to better connect these observations to the reaction mechanism.



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Wednesday, June 4<sup>th</sup> | 10:00

## TEM and STEM Imaging of Radiation-Sensitive Samples

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Beam damage to biological specimens is more troublesome in the TEM than in x-ray imaging (where the spatial resolution is more modest) despite the stronger diffraction signal provided by electrons [1]. One solution has been to treat tissue (and other) specimens with heavy-metal stain but the preference is to use unstained samples and phase contrast, while cooling the sample to reduce damage (cryo-EM).

Whereas the electron optics of a TEM can provide atomic-scale contrast, the image resolution for a beam-sensitive sample is usually limited by the effects of radiolysis. The signal/noise ratio (SNR) in the image is then determined by beam-electron shot noise and the damage-limited resolution is given [2] by

$$\text{DLR} = (\text{SNR}) C^{-1} [(DQE) F D_c]^{-1/2} \quad (1)$$

Optimizing resolution involves paying attention to each term in Eq. (1). SNR is usually taken as 3 or 5 (the Rose criterion) but a large improvement is possible if there are multiple copies of the same object, as in diffraction imaging or single-particle analysis (SPA). The image contrast  $C$  is high in dark-field mode but for thin samples the electron-collection efficiency  $F$  is low. Phase contrast is efficient but is problematic for thicker samples, as required for most tomographic (3-dimensional) imaging. The detective quantum efficiency (DQE) of the electron detector can be maximized by using direct recording, rather than employing a scintillator.

The characteristic dose  $D_c$  represents the maximum fluence that the specimen can withstand before the structure being observed is degraded.  $D_c$  can be increased by cooling the sample with liquid nitrogen or liquid helium [3] or by coating it with an evaporated layer, and graphene encapsulation seems to be even more effective

Dose rate seems to have little effect on the radiolysis damage to organic specimens, except for secondary effects (mass loss) at a low specimen temperature and at the high dose rates ( $>10^{15}$  Gy/s) possible in scanning-mode TEM (STEM). Unlike XFEL x-ray imaging, it will likely remain impossible to outrun the primary stage of damage with electrons, due to their electrostatic repulsion. Earlier reports of two-fold damage reduction using femtosecond electron pulses have recently been disputed [4].

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## Extending the reach of single-particle cryoEM

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Ten years on from the “resolution revolution”, molecular structure determination using electron cryomicroscopy (cryoEM) is poised in 2025 or early 2026 to surpass X-ray crystallography as the most used method for experimentally determining new structures [1]. But the technique has not reached the physical limits set by radiation damage and the signal-to-noise ratio in individual images of molecules. By examining these limits and recent work on radiation damage to biological molecules at different temperatures [2,3] and energies [4,5], I will identify opportunities for extending the application of single-particle cryoEM to smaller, larger and more difficult structures, and into specimens taken directly from vitrified cells. This will help guide technology development to continue the exponential growth of structural biology in the coming decade.

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## Perspectives and limitations in high-resolution cryo-EM

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Single particle cryo-EM has become a powerful technology in structure determination of macromolecular complexes. The technique benefits from the decades of investment in the development of high-end electron microscopy hardware, powerful computational hard- and software and the fact that samples do not need to be crystalized. Over the past decade, the number of structures solved by cryo-EM has consequently increased and soon a point will be reached when more structures will be solved in academia by cryo-EM than by X-ray crystallography. Simultaneously, the maximum resolution limit of the technology has been improved and at least for the model protein apoferritin even atomic resolution has been achieved [1].

In spite of this success story, the mean resolution of single particle cryo-EM structures deposited in the database (EMDB), is still >4 Angstrom even until today. The important question is therefore, why can this technology potentially obtain even atomic resolution structures and what are the limiting factors that prevent the user from obtaining this level of resolution on a regular basis?

A major limiting factor in cryo-EM is the biochemical quality of a purified macromolecular complex. Macromolecules are known to be prone to suffer in quality during biochemical purification but also during electron microscopical grid preparation and often it isn't even an easy task to distinguish between the two effects. Other limitations can be attributed to electron optical limitations but also limitations in computational image processing.

Achieving routine atomic resolution depends on parallel improvements in all three pillars of the technology. For example, even a small amount of sample degradation during biochemical purification or grid preparation can negate the gains made by state-of-the-art electron microscopes. Similarly, poor electron optics can limit the usefulness of pristine samples. Overcoming these challenges requires a holistic approach: refining biochemical workflows to preserve macromolecular integrity, optimising electron optics to minimise imaging artefacts, and developing adaptive software pipelines to extract maximum information from complex datasets.

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## A Physical Theory For Cryo-EM at Liquid-Helium Temperatures

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The benefits of reducing the data collection temperature for electron cryomicroscopy (cryo-EM) from liquid-nitrogen temperatures to liquid-helium temperatures have been debated over many years. A physical theory of dose-dependent information loss in cryo-EM was presented for imaging vitrified aqueous biological specimens at liquid-nitrogen temperatures [1], but extending this to liquid-helium temperatures is needed. Previously, it was demonstrated that there is a 1.2–1.8x reduction in radiation damage for 2D protein crystals when imaging at temperatures near liquid helium [2]. Unfortunately, lowering specimen temperatures for cryo-EM of macromolecules embedded in vitreous ice has consistently proven to be no better than liquid-nitrogen temperatures and is often worse [3]. We aimed to determine whether the reduction in radiation damage measured in 2D crystals extends to single-particle cryo-EM and, if so, what else could be limiting data quality.

Consequently, we investigated several dose-dependent physical phenomena that could limit single-particle cryo-EM data quality: radiation damage, microscopic charge fluctuations, charge accumulation, pseudo-Brownian motion of water, mass loss, hydrogen bubbling, and beam-induced motion. We found that radiation damage is reduced by a similar amount for single-particle cryo-EM as was measured by 2D crystallography. We demonstrate that the reduction in data quality is likely caused by beam-induced motion, with all other physical phenomena that we measured being either unchanged or not sufficient to cause a reduction in image quality at lower specimen temperatures. Using novel specimen supports, we have been able to eliminate this beam-induced motion and determined cryo-EM structures at liquid-helium temperatures where every frame carries more information compared to the equivalent at liquid-nitrogen temperatures [4]. Alongside the development of new TEMs capable of operating at temperatures below liquid-nitrogen, this theory will enable cryo-EM to resolve smaller molecules than is currently possible.

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## Cryo-EM structure of photosystem II reveals damages caused by commonly used electron doses in imaging

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Photosystem II (PSII) plays a critical role in water-splitting and oxygen evolution. X-ray crystallography has elucidated its atomic structure and structures of several intermediate states. However, these structures are in the crystalline state, and the structure of the final state remains unresolved. In this study, we analyzed the structure of PSII in solution at a resolution of 1.95 Å using single-particle cryo-electron microscopy (cryo-EM). The obtained structure is similar to the crystal structure, but regions susceptible to redox state changes exhibited electron beam damages at high doses. By reducing the number of movie frames of electron micrographs from 50 to 2 to lower the beam dose, the damages were minimized while the resolution was comparable (Fig. 1). I will discuss the details of the electron beam-induced damages and their minimization in this presentation.

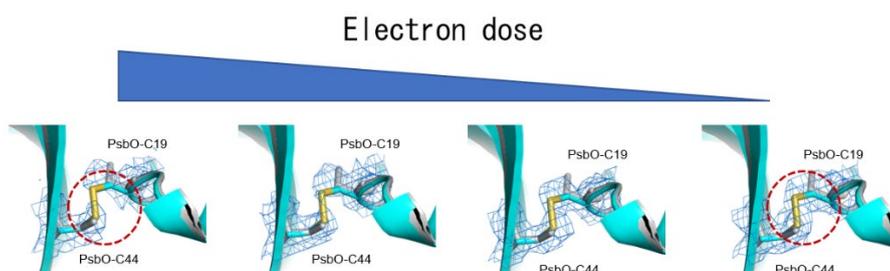


Fig. 1 Reduction of disulfide bond by electron beam and its minimization by reducing the beam dose.

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Wednesday, June 4<sup>th</sup> | 13:00

## Radiation damage in recent MicroED measurements

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Microcrystal electron diffraction (MicroED) involves collecting a sequence of diffraction images from a continuously rotating microcrystal in a transmission electron microscope. Due to the small size of these crystals, the resulting diffraction patterns typically feature weak, low-intensity spots. Recent advances in direct electron-counting detectors have significantly improved signal detection, enabling high-quality data collection at lower electron fluence. This not only shortens acquisition times but also mitigates radiation damage to the sample. Additionally, the introduction of energy filtering has enhanced the accuracy of high-resolution reflection integration without increasing the dose delivered to the sample. In this presentation, we will review early results from radiation damage studies on cryo-cooled crystals of model proteins and highlight recent methodological improvements in MicroED.

Wednesday, June 4<sup>th</sup> | 16:30

## High-dose effects in high-resolution X-ray microscopy of soft materials

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High-resolution X-ray microscopy is used as a complementary approach to electron microscopy for non-destructive imaging of soft materials, including biological tissues, e.g. frozen hydrated [1] or heavy metal stained resin-embedded [2] brain tissues. For this purpose, photon energies above about 2 keV are used to penetrate tissues of about 100 micron thickness or more, for which samples exhibit a very low contrast. To overcome this challenge, phase-contrast hard X-ray microscopy methods are typically used in synchrotron beamlines, reaching a resolution of about 100 nm. The available coherent flux and the changes in the sample structure due radiation are among the main challenges to improve spatial resolution in hard X-ray microscopy of soft materials. Next generation synchrotron sources provide high brilliance, which offers a great opportunity to improve resolution. However, this will require the development of new approaches to mitigate the effects of radiation in soft materials.

Here, we present our experience when applying ptychographic X-ray computed tomography (PXCT) [3] to soft materials. In polymer samples or resin-embedded biological tissues, we observe deformations, such as expansion or contraction of the sample, and mass loss above a certain X-ray dose exposure [4]. For samples that deform during acquisition, we apply a non-rigid tomographic reconstruction to recover the original 3D structure of the specimen [5]. For resin-embedded biological tissues, we have identified a resin which is more resistant to hard X-ray radiation compared to standard epoxy resins used for EM [4]. Finally, we explore acquisition strategies and sample preparation protocols that minimize the effect of radiation.

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## Coherent X-rays Reveals Radiation-Induced Dynamics in Hydrated Proteins

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Radiation damage remains a central challenge in structural biology, particularly when probing soft, disordered materials like hydrated proteins. In this presentation, I will discuss recent advances using X-ray Photon Correlation Spectroscopy (XPCS) to study radiation-driven dynamics in both hydrated protein powders [1] and dense protein solutions [2,3]. At cryogenic temperatures, we investigate lysozyme powders and find that X-ray exposure induces nanoscale stress relaxation, revealing a temperature-dependent transition around 227 K associated with enhanced dynamical heterogeneity. These results highlight how radiation can stimulate out-of-equilibrium processes in supercooled, granular biomaterials. At ambient temperatures, using megahertz XPCS at the European XFEL, we probe antibody and ferritin solutions as a function of X-ray dose and dose rate. Our measurements capture anomalous diffusion and aggregation dynamics, which are influenced by both hydrodynamic and direct protein interactions. Modeling these effects allows us to disentangle intrinsic molecular behavior from radiation-induced perturbations. Together, these studies underscore the dual role of coherent X-rays as both probe and stimulus, offering critical insights into how radiation impacts biological materials under experimentally relevant conditions. This understanding is essential for optimizing measurement strategies in next-generation X-ray facilities and for minimizing radiation effects in XPCS studies of biological samples.

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## Radiation damage in time-resolved X-ray solution scattering experiments

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Time-resolved pump-and-probe X-ray solution scattering experiments can probe kinetics and dynamics of biological macromolecules in real time and under nearnative conditions. A concern is radiation damage to the sample. One approach to reduce radiation damage is to use mechanical devices to probe only with short X-ray pulses. We used this approach to monitor intermediate states in calcium transport at beamline ID09 at the ESRF 1,2. However, such experimental designs are delicate and typically only available at dedicated time-resolved synchrotron beamlines, which limits availability. An alternative approach is to use detector readout to obtain the temporal resolution, which requires careful characterization of radiation damage. We developed such an approach at the CoSAXS beamline at MAX IV Laboratory and tracked kinetics and structural dynamics of the enzyme adenylate kinase 3.

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Thursday, June 5<sup>th</sup> | 9:00

## Ultrafast structural changes in myoglobin: influence of pump laser fluence

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The high-intensity femtosecond pulses generated by X-ray free-electron lasers enable pump-probe studies of electronic- and nuclear changes during light-induced reactions. On time scales from femtoseconds to milliseconds and for a variety of biological systems, time-resolved serial femtosecond crystallography (TR-SFX) has provided detailed structural data on processes such as light-induced isomerization, breakage or formation of chemical bonds and electron transfer. However, to date, most if not all ultra-fast TR-SFX studies have employed such high pump laser energies that nominally, several photons were absorbed for each chromophore. As such multiphoton absorption processes may force the protein response into nonphysiological pathways, this is of considerable concern as it poses the question whether this experimental approach allows valid inferences to be drawn about biological processes, which are likely single-photon.

Here we describe an ultrafast pump-probe SFX study of the photodissociation of carboxymyoglobin, which shows that different pump laser fluences result in strikingly different dynamics. In particular, these concern the mechanistically important coherent oscillations of the Fe-CO bond distance (predicted by recent quantum wavepacket dynamics) which are seen to depend strongly on pump laser energy. While our results confirm both the feasibility of performing TR-SFX pump probe experiments in the linear photoexcitation regime, they also show the necessity of doing so. We propose this to be a starting point for the reassessment of the design and interpretation of ultrafast TR-SFX pump probe experiments, to ensure any emergent insights are biologically relevant.

## Time-resolved serial femtosecond crystallography on the fluorescent protein rsEGFP2-V151A in different photon excitation regimes

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Time-resolved serial femtosecond crystallography (TR-SFX) enables the visualization of ultrafast structural changes in crystalline macromolecules [1]. For light-sensitive proteins, optical excitation lasers represent a convenient means to trigger the reaction. Generally, high excitation-laser fluences are used to maximize light-induced features in the Fourier difference electron density maps [2,3]. However, these fluences generally correspond to nominally more than one absorbed photon per chromophore on average, significantly increasing the risk of unwanted multiphoton effects that convolute with the functionally-meaningful single-photon process of interest [4]. Hence, the choice of excitation laser fluence is a topic of intense discussion in the TR-SFX field [5-7] and a first systematic study on myoglobin indeed evidenced different CO dissociation mechanisms in the single- and the multi-photon regimes [4].

Here, TR-SFX experiments were conducted on the V151A variant of the reversibly photoswitchable fluorescent protein rsEGFP2 [8]. The off- (trans chromophore) to on-state (cis chromophore) photo-switching process was probed at two different time delays (1 and 500 ps) following 150-femtosecond excitation at low, medium and high fluences (0.05, 0.15 and 0.5 mJ/mm<sup>2</sup> at the Gaussian peak, respectively) corresponding nominally to 0.8, 2, and 8 absorbed photons per chromophore on average. At the high fluence, only a marginal further increase is observed in light-induced Fourier difference electron density peaks compared to the medium fluence, and the expected cis conformer is either absent (1 ps) or occupied below expectation (500 ps). Fluence-dependent time-resolved absorption spectroscopy suggested a chromophore radical species only formed at the high fluence. Our findings suggest that multi-photon induced radical formation at high fluence alters the functional photoisomerization process in rsEGFP2.

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## Structural effects of high laser power densities on an early bacteriorhodopsin photocycle intermediate

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PSI

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Time-resolved serial crystallography at X-ray Free Electron Lasers offers the opportunity to observe ultrafast photochemical reactions at the atomic level. The technique has yielded exciting molecular insights into various biological processes including light sensing and photochemical energy conversion. However, to achieve sufficient levels of activation within an optically dense crystal, high laser power densities are often used, which has led to an ongoing debate about the extent to which photodamage may compromise the interpretation of the results. Here we compare time-resolved serial crystallographic data of the bacteriorhodopsin K-intermediate collected at laser power densities ranging from 0.04 to 2493 GW/cm<sup>2</sup> and follow energy dissipation of the absorbed photons logarithmically from picoseconds to milliseconds. Although the effects of high laser power densities on the overall structure are small, in the upper excitation range we observe significant changes in retinal conformation and increased heating of the functionally critical counterion cluster. We compare light-activation within crystals to that in solution and discuss the impact of the observed changes on bacteriorhodopsin biology.

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*Thursday, June 5<sup>th</sup> | 11:00*

## Mapping the Conformational Landscapes of Human Kinases with Multi-Temperature X-ray Crystallography

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*Mike Thompson  
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Protein kinases are a large family of enzymes that regulate diverse cellular processes by transferring phosphate groups from ATP to their protein substrates. The enzymatic kinase domain (KD) represents a conserved fold, and KD phosphorylation, as well as interactions with cis-regulatory elements and other allosteric effectors, modulates the populations of catalytically active and inactive states present in the KD conformational ensemble. In this presentation, I will briefly describe two studies where multi-temperature X-ray crystallography has provided new insight into the conformational landscapes of two human kinases. In one study, we used determined crystal structures of CDK2, a kinase that regulates the cell cycle and is considered a „white whale“ of drug discovery, across a broad range of temperatures from cryogenic to physiological, to identify inactive conformations that could be targeted to create more selective inhibitors that avoid off-target effects. In the other study, we determined crystal structures of CLK1 across a narrow range of physiological temperatures to understand how this kinase acts as an acute body temperature sensor. Because these studies involve extensive data collection at non-cryogenic temperatures, careful consideration must be given to mitigating the effects of radiation damage.

## Exploring Ligand-Protein Interactions at Multiple Temperatures Using Macromolecular Crystallography

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Cryogenic temperatures may introduce artefacts that limit the understanding of protein dynamics, crucial to their biological functions. To address this, we developed a room-temperature (RT) X-ray crystallographic method that captures movie-like structural snapshots triggered by temperature [1]. This method revealed binding-mode changes of TL00150, a 175.15 Da fragment, in endothiapepsin. Building on this, we further developed Cryo2RT, a high-throughput RT data-collection method using cryo-cooled crystals, which leverages the cryo-crystallography workflow [2]. This method has been applied to endothiapepsin with four soaked fragments, thaumatin, and SARS-CoV-2 3CLpro, Cryo2RT uncovered distinct ligand-binding modes at RT, not seen at cryogenic temperatures. To minimize radiation damage, X-ray doses were controlled below 500 kGy, a threshold considered safe for both cryo and RT crystallography. Despite similar doses, RT datasets showed slightly lower resolution and higher B-factors (30–40 Å<sup>2</sup> vs. ~20 Å<sup>2</sup> at cryo), likely due to increased atomic motion at RT. These findings provide insights into structural interpretation at RT and highlight Cryo2RT's potential for fragment-based screening and studying temperature-dependent dynamics.

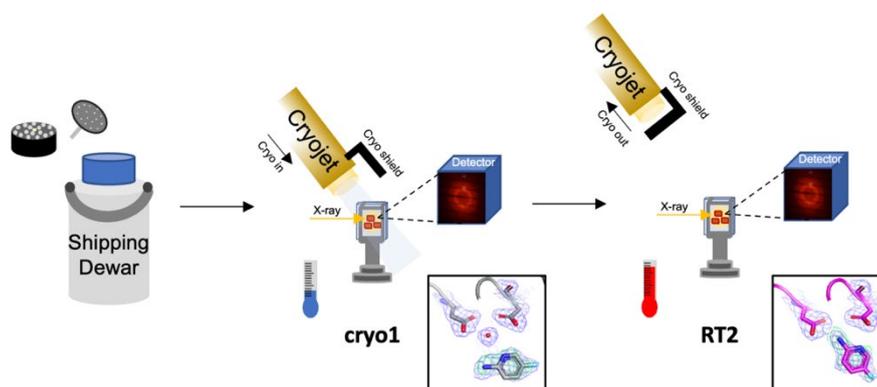


Figure 1 The Cryo2RT workflow.

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## Posters

The posters are viewable for the duration of the workshop outside the Auditorium.

The poster session will take place after dinner on day 1, Tuesday 3rd of June, from 20:00 – 21:30.

#	Presenter	Title
1	Pauline van Deursen	Streamlining Graphene Liquid Cell Preparation: VitroTEM's Naiad System
2	Adam D. Crawshaw	A comparison of an X-ray diffraction and electron diffraction experiment from a single protein microcrystal lamella
3	Yelyzaveta Pulnova	HATRX for radiation damage study
4	Maria C. Kokkinidou	Enhanced cryogenic cooling for imaging of large samples by X-ray microscopy
5	Gerhard Hofer	Continuous Serial Electron Diffraction (SerialED) for High-Quality Protein Structures
6	Hugh Wilson	Reducing radiation damage from cryoEM maps
7	Pedro Nunes	HeXI: The High-energy Electron Xtallography Instrument
8	Tobias Weinert	Photon time efficiency: a simple metric for evaluating X-ray Sources
9	Antoine Royant	TR-icOS: a pump-probe spectroscopic instrument for the preparation of TR-SSX or TR-SFX experiments
10	Samuel Foster	Identification of X-ray induced cysteine oxidation within the Protein Data Bank
11	Alexander Shtyrov	Modelling of radiation damage as a Gaussian process
12	Shervin Nia	Mechanistic Analyses of Radical Propagation in Protein CryoEM
13	Spencer K. Passmore	Heavy-element damage seeding in proteins under XFEL illumination: How 10% NaCl can double the dose.
14	Ronald Rios-Santacruz	X-ray induced substrate decarboxylation in fatty acid photodecarboxylase observed by macromolecular and serial synchrotron crystallography
15	Jannik Wiebe	Exploring Specific Radiation Damage to Halogenated Ligands in Crystal Structures Collected at Cryogenic and Room Temperature
16	Edward Snell	Understanding the Mechanism and Health Consequences of Low-Dose Radiation at a Molecular Level

## Streamlining Graphene Liquid Cell Preparation: VitroTEM's Naiad System

*Pauline van Deursen<sup>1\*</sup>, Hans Radhoe<sup>1</sup>, Sheetal Bhardwaj<sup>1</sup>*

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Encapsulation in Graphene Liquid Cells (GLCs) enhances the beam resistance of samples in electron microscopy through three main mechanisms: added electrical conductivity, electrical conductivity, and the radical scavenging behavior exhibited by graphene[1]. These properties make graphene, and in particular GLCs – in which the sample is interfaced with a graphene layer on both sides - a much sought-after sample carrier.

The implementation of graphene liquid cells in electron microscopy has been hampered by difficulty in handling this ultrathin 2D material. Moreover, inferior quality and cleanliness have been common experiences to users of graphene from commercial sources.

This poster presents VitroTEM's Naiad system, a revolutionary approach to GLC preparation. By assembling two monolayers of graphene on a single standard TEM grid, the Naiad system rapidly constructs GLCs, encapsulating liquid samples for imaging[2]. Our poster showcases images the effective stabilization of protein crystals in micro-crystal electron diffraction. GLC help to conserve the hydrated structure of the crystal, resulting in efficient data collection even at room temperature.

The Naiad system simplifies GLC assembly, enabling researchers to focus on sample imaging rather than grappling with graphene preparation. This poster emphasizes the Naiad system's potential to accelerate discoveries in diverse fields reliant on high-resolution imaging of liquid-phase as well as cryo samples.

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## A comparison of an X-ray diffraction and electron diffraction experiment from a single protein microcrystal lamella

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X-ray diffraction (XRD) of microcrystals is signal-to-noise limited by due to the inherently weak diffraction. Therefore, it is key that the beamline instrumentation and the sample itself introduce minimal noise. The VMXm beamline, at Diamond Light Source, has been optimised for maximising the S:N in experiments with a variable focus high-energy (>20 KeV) X-ray beam, with in-vacuum endstation and the use of low background cryoTEM grids for crystal mounting [1], [2]. This has allowed VMXm to collect high-resolution rotation data from single crystals measuring ~1.2  $\mu\text{m}$  which were only previously tractable using an X-ray Free Electron Laser [3]. This has pushed the amenable sample envelope at synchrotrons to new dimensions and perhaps near to the practical limit of XRD. Indeed, simulations have predicted the limit to be ~0.5  $\mu\text{m}$  thick in the case of lysozyme, assuming photoelectron escape [4].

Electron diffraction (ED) is frequently used to measure diffraction data from submicron crystals. Many samples which are too thin for XRD are often too thick for ED using the currently available electron beam energies (<300 keV) and hence require thinning by focussed ion beam milling (FIB). In addition to determining structures from nanocrystals, ED provides Coulomb potential data which are complementary to that obtained with XRD. As such ED data may be necessary to answer particular scientific questions.

In this work we present data from cubic human insulin crystals that have been thinned by FIB milling from ~10  $\mu\text{m}$  to various submicron thicknesses. 200 kV ED data were then collected from these lamellae before XRD data were measured from the same lamellae using VMXm. It was possible to obtain a complete XRD dataset to 2.45  $\text{\AA}$  using a 1.68  $\mu\text{m}^3$  illuminated volume and a 2.04  $\text{\AA}$  ED dataset from the same 0.25  $\mu\text{m}$  lamella. We have demonstrated that the data quality is comparable between ED and VMXm from the same crystal, while giving an opportunity to directly compare X-ray and electron derived maps. This includes the comparison of the radiation damage each experiment imparts on the sample [5] as well as the information content [6]. This work indicates that the usable sample envelope for synchrotron X-rays extends to much thinner samples than had been previously thought. It is also the first demonstration of ED and XRD measured from the same crystal volume enabling direct comparison of X-ray and electron derived data. Ultimately, the work will inform the design and use of high energy (MeV) ED instruments such as HeXI and how those can be complemented by XRD derived information from beamlines such as VMXm.

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## HATRX for radiation damage study

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Time-resolved diffraction is becoming an established technique in large-scale facilities such as synchrotrons and XFELs. Alternative sources such as plasma X-ray source (PXS, Fig. 1A) [1] offer attractive pulse durations of units of picoseconds with lower operational cost than XFEL facilities. However, the downsides of PXS sources are the unstable flux and comparatively low brilliance. The latter may be overcome by a stroboscopic [2] or multiplexing [3] approaches, however, the majority of macromolecular samples are radiation sensitive and undergo irreversible reactions limiting the application of stroboscopic data collection.

Hadamard Time-Resolved Crystallography (HATRX) is a multiplexing technique, where diffraction is measured as an ensemble of individual time points (Fig. 1B). The individual time-resolved data are then reconstructed using the Hadamard transform. Multiplexing requires the ability to measure diffraction at distinct time-points utilising either detector-gating or a pulsed source. We present initial results demonstrating radiation damage during a HATRX experiment using the detector-gating technique at beamline I24 (Diamond Light Source). The necessary pulse-sequences may be introduced onto the driving laser of the PXS, which is advantageous over detector gating since the sample is only exposed to X-rays when data are being collected. We discuss the potentials and pitfalls of the PXS for HATRX studies.

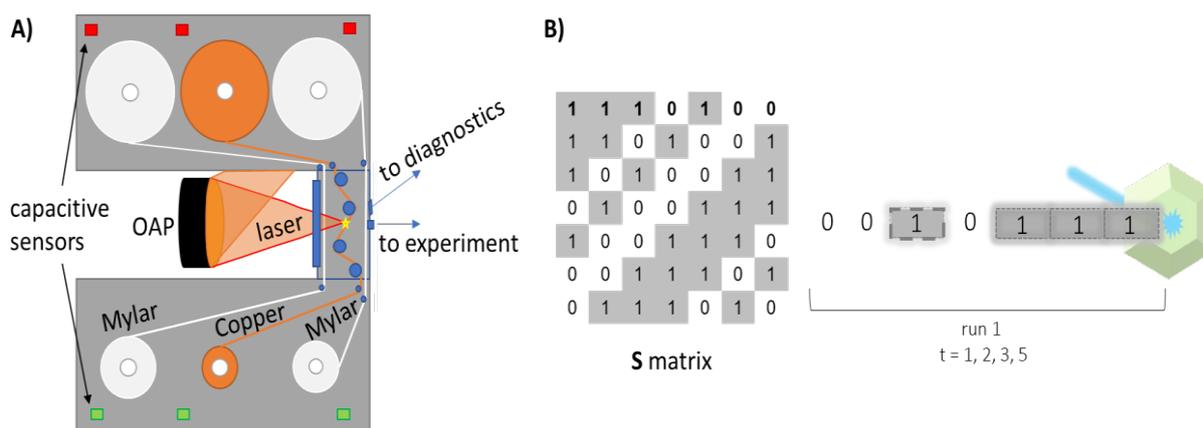


Fig.1: A) Scheme of PXS B) The X-ray probing sequence for the HATRX is structured as a matrix, where each row represents an individual measurement run. In this matrix, a value of one indicates a beam- ON state, while a value of zero signifies a beam-OFF state.

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## Enhanced cryogenic cooling for imaging of large samples by X-ray microscopy

*Charles Wood<sup>1</sup>, Atousa Moayedi<sup>1</sup>, Thomas Renshaw<sup>2</sup>, Maria C. Kokkinidou<sup>2</sup>, Jonathan Shaxted<sup>2</sup>, Alex Renshaw<sup>2</sup>, Lucjan Pajdzik<sup>2</sup>, Alan Hodgson<sup>2</sup>, Adriana L. Klyszejko<sup>2</sup>*

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Developments in instrumentation including cryogenic sample environments provide platforms for multimodal tomography application. To increase cryogenic cooling capabilities for large samples, we developed Cryostream 1000 Wide Nozzle. It provides a much larger gas volume when compared to the Standard model and facilitates vitrification of samples up to 3 mm in diameter. The Wide Nozzle has been successfully used to cool large crystals and biological specimens for X-ray and neutron diffraction and scattering studies, and hard X-ray microscopy tomography.

We present the development of a lab-based cryogenic hard X-ray imaging system (microCT), designed for sub-micron resolution of biological specimens with reduced need for contrast agents which might affect biological systems.

Utilising the Oxford Cryosystems Cryostream 1000 Wide Nozzle, in conjunction with a Zeiss Versa 610 X-ray Microscope, we demonstrate that delicate biological samples, otherwise degraded under lab X-ray imaging conditions, can be successfully imaged under stable cryogenic conditions over a period of several hours.

Whilst further tests are required, we observed enhancement of absorption contrast and signal-to-noise ratio by reduction of thermal scattering. We visualized fine structural details without heavy metal staining and other associated artefacts that often arise in conventional X-ray imaging.

To map thermal gradients within the cryogenic stream, we used a thermal diode providing precise temperature data across the gas column, helping to guide future optimisation of cooling dynamics. These insights are important for ongoing work aimed at expanding the Cryostream's application to a range of materials, with potential implications for fields like biomedicine, tissue preservation, tomography, aerospace, energy materials, and heritage artefacts.

This cryogenic X-ray system, therefore, represents a significant step towards lab-based cryogenic X-ray imaging, and further developments will focus on quantifying the contributions of X-ray absorption and scatter components at cryogenic temperatures, broadening the system's capabilities for multi-modal microscopy and in situ analyses.

Keywords: X-ray microscopy, hard X-ray, microCT, multimodal microscopy, sample environments, cryogenic cooling, sample preservation, vitrification, tomography, open flow cooling, Cryostream 1000, Cobra, Wide Nozzle

## Continuous Serial Electron Diffraction (SerialED) for High-Quality Protein Structures

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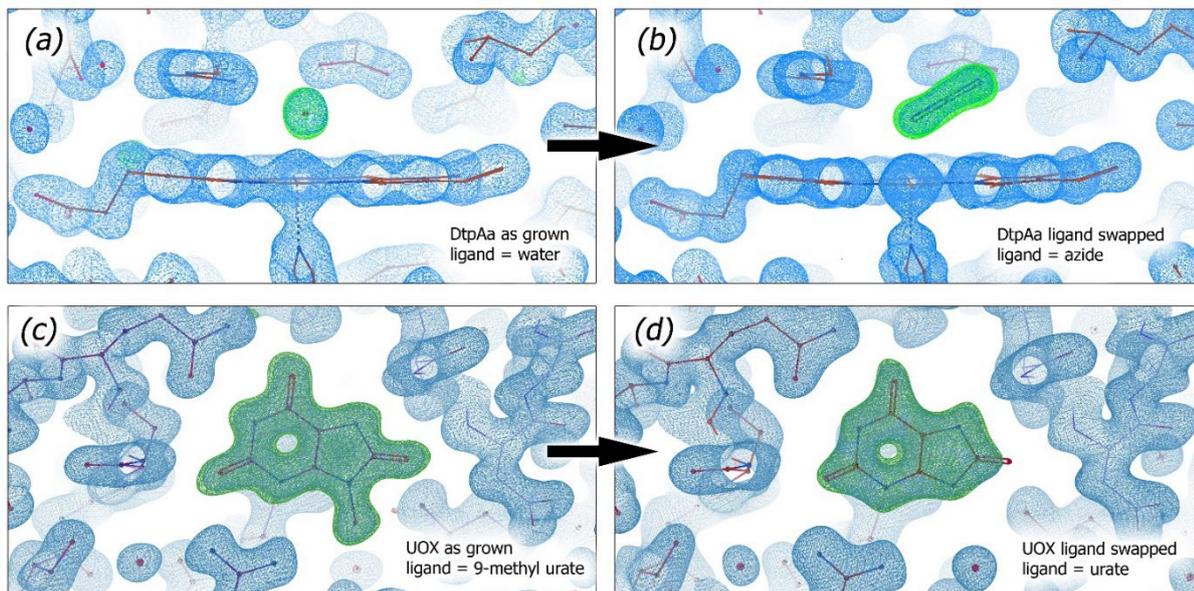
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Serial electron diffraction (SerialED) promises several advantages over traditional rotational data collection methods such as microED/3DED. By distributing radiation over multiple crystals, SerialED achieves higher signal-to-noise ratios, which lead to higher resolution structures and significantly reduced radiation damage. This improvement is particularly beneficial for addressing site-specific questions such as small ligand binding, hydrogen positions, and charge states, which are often challenging to elucidate with conventional methods.

Our latest development in the field, continuous serial electron diffraction (SerialED) provides resolutions only limited by the order of the crystals, shows no sign of radiation damage and can be collected on nearly any TEM. Together with a method to produce dense slurries of nano-crystals from well behaving proteins and an easy manual grid preparation this enables ligand screening with exceedingly short soaking times (seconds). This also provides enzyme/substrate complex structures in a straightforward manner. By also simplifying the setup and data processing steps, we aim to democratize the use of SerialED, enabling more laboratories to leverage this powerful technique for their research.



*Figure 1- On-grid ligand exchange visualised by continuous SerialED omit maps All 2Fo-Fc maps are non-filled and drawn at 1.5 rmsd (blue), Fo-Fc maps at 4 rmsd (green,red)*

## Reducing radiation damage from cryoEM maps

*Hugh Wilson, Alexander Shtyrov, Jude Short, Katerina Naydenova Daria Slowik, Shaoxia Chen, Jade Li, Greg McMullan, Garib Murshudov, Richard Henderson, Christopher J. Russo*

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CryoEM is now a powerful tool for biomolecular structure determination. However, nearly all cryoEM maps to date provide records of a damaged structure of the molecule. Movement of the sample during the first few  $\text{e}/\text{\AA}^2$  of an exposure blocks access to the structural information when the molecule is least damaged. As a result, features that are radiation sensitive are not well-resolved in the final map. Here we present work to enable the elimination of radiation damage from cryoEM maps. We will outline the development of small-hole cryoEM grids which suppress sample movement during irradiation. This provides access to the start of the exposure where the structure is least affected by radiation damage and enables extrapolation back to a map at zero dose. Damage-free maps promise access to new types of structural information, such as charge states, that would be beneficial for interpreting mechanism and biological function. We believe this work will be of broad interest to the radiation damage community.

## HeXI: The High-energy Electron Xtallography Instrument

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The High-energy Electron Xtallography Instrument (HeXI), currently under construction at Diamond, aims to investigate the use of Mega-electron-volt (MeV) electrons for macromolecular structure determination, thereby broadening the range of samples suitable for electron diffraction. Funded by the Wellcome Trust “Electrifying Life Sciences” grant and Diamond Light Source, the HeXI project will leverage the increased penetration of Mega-electron-volt (MeV) electrons to bridge the crystal size gap between electron and X-ray scattering, enabling the determination of structures from crystals ranging between 300 nm and 3  $\mu\text{m}$ .

A tunable electron source, operating between 100 kV and 1 MeV, will be used to: explore improvements in data quality arising from reduced dynamical scattering at higher incident electron energies, and to investigate the interplay between sample thickness and incident electron energy in damage mechanisms. HeXI will use advanced goniometry developed at Diamond for macromolecular X-ray crystallography to reduce measurement geometry instabilities and enhance overall data fidelity.

HeXI, depicted in Figure 1, will offer data collection under three modalities:

- Three-dimensional electron diffraction (3DED): Structure determination of pharmaceutically relevant molecules without further purification/crystallisation.
- Cryo-3DED/microED: Structure determination of frozen-hydrated protein crystals which are too small for traditional X-ray macromolecular crystallography.
- SerialED: Time-resolved (ms to  $\mu\text{s}$ ) structure determination of protein crystals.

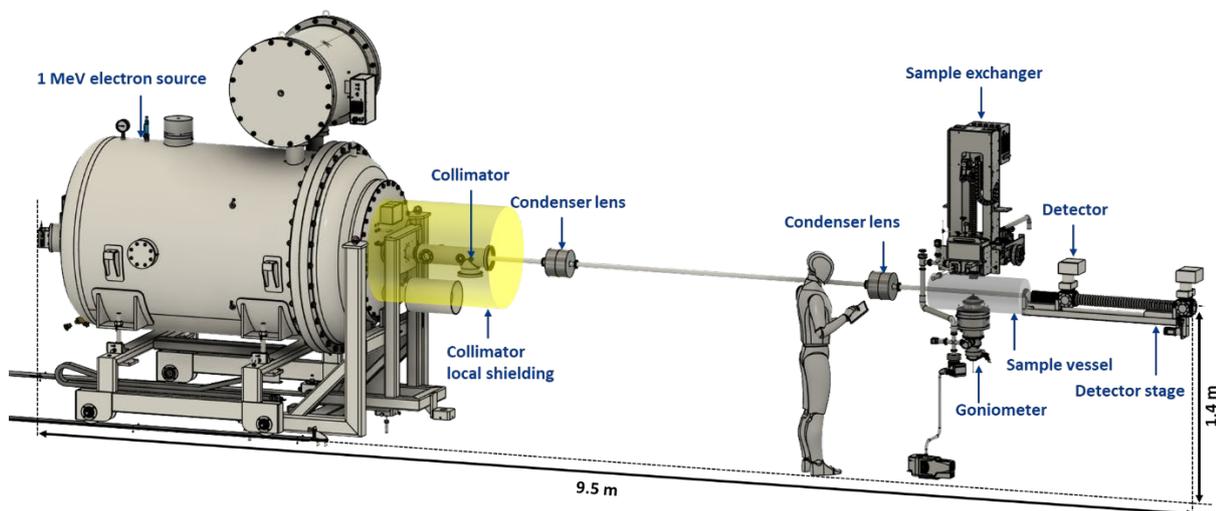


Figure 1. CAD model of the HeXI instrument.

## Photon time efficiency: a simple metric for evaluating X-ray Sources

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The progress of structural biology has been closely linked to the development of increasingly powerful X-ray sources. In recent years, serial crystallography has revolutionized protein structure determination, particularly in time-resolved studies that require room-temperature data collection. A critical but often overlooked factor in the success of these experiments is photon time efficiency—the rate at which useful diffraction data can be collected within a given timeframe.

This poster evaluates photon time efficiency across different X-ray sources, considering the constraints imposed by radiation damage, sample delivery methods, and beam characteristics. The efficiency of serial crystallography depends on maximizing the number of collected diffraction patterns while ensuring an optimal dose for each crystal.

The diffraction-before-destruction principle gives X-ray free-electron lasers a distinct advantage in maximizing photon time efficiency. However, diffraction-without-destruction approaches offer unique benefits, particularly for time-resolved serial crystallography. This comparison highlights key trade-offs in optimizing experimental outcomes across different X-ray facilities.

## TR-*icOS*: a pump-probe spectroscopic instrument for the preparation of TR-SSX or TR-SFX experiments

*Antoine Royant*<sup>1,2</sup>, *Samuel Rose*<sup>2</sup>, *Nicolas Caramello*<sup>1</sup>, *Thierry Giraud*<sup>2</sup>, *Philippe Jacquet*<sup>1</sup>, *Po-Hsun Wang*<sup>3</sup>, *Sergei Bukhdruker*<sup>1</sup>, *Yuhei Hosokawa*<sup>3</sup>, *Manuel Maestre-Reyna*<sup>3</sup>, *David Flot*<sup>2</sup>, *Christoph Mueller-Dieckmann*<sup>2</sup>, *Sylvain Engilberge*<sup>1</sup>

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Following up on the development of time-resolved serial femtosecond crystallography (TR-SFX) at free-electron lasers, the technique has been rejuvenated at synchrotrons (and renamed TR-SMX, or TR-SSX), resulting in the design of dedicated beamlines, on which the mechanism of photoactive proteins can be studied with a microsecond time resolution using pump-probe schemes. In order to identify relevant time delays, time-resolved spectroscopic experiments directly performed on protein crystals are desirable. To this end, at the *icOS* Lab of the ESRF [1], we have built an instrument based on a nanosecond laser and a xenon flashlamp to rapidly record pump-probe spectra from single crystals or slurries of microcrystals with delays ranging from microseconds to seconds, at various laser pulse energies [2]. The resulting information should help fine-tune experimental laser and delay parameters for a successful TR-SX experiment. We have first commissioned the instrument using crystals of the membrane proton pump bacteriorhodopsin and showed that high laser fluences distorted the photocycle of the protein. We then used it to visualize the key protonation step of the flavin radical occurring *in crystallo* during the photoreaction of an animal-like cryptochrome, whose associated structural changes have been observed at the XFEL SACLA between tens of nanoseconds and hundreds of milliseconds [3].

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## Identification of X-ray induced cysteine oxidation within the Protein Data Bank

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X-ray induced photoreduction of macromolecular structures has been well reported, with the accompanying site-specific radiation damage occurring in a predictable order. Metal containing centres are reduced first, followed by disulphide reduction, decarboxylation of glutamate and aspartate residues and then increased side chain mobility [1]. Photoreduction of the bulk solvent also occurs which contributes to global radiation damage, identifiable in data processing statistics. In a recent study into the structure of a glyceraldehyde 3-phosphate dehydrogenase (GAPDH) from a human pathogen, the active site cysteine was noted to be modified to a sulfinic acid (R-SO(OH)). GAPDH is a core metabolic enzyme, involved in ATP and pyruvate generation by catalysing the reversible oxidative phosphorylation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate [2]. An oxidised active site cysteine is incompatible with the current reaction mechanism for GAPDHs [3]. This type of modification has been noted previously for GAPDHs and has been attributed to post-translational modifications by reactive oxygen species [4, 5]. The same modification, however, has been noted for other oxidoreductases and postulated to be a form of site-specific radiation damage, where the activated cysteine is oxidised by hydroxyl radicals formed in the bulk solvent [1, 6]. In this work we have mined the PDB for all X-ray structures of GAPDHs and devised a workflow to identify damaged cysteines. Of the 225 structures, 68 were identified as damaged. The damage appears to be decoupled from conventional metrics for identifying specific radiation damage (e.g., Bnet-percentile [7]) and is independent of data collection source or temperature. The method implemented is highly sensitive to damaged cysteines and is effective in screening large datasets. This work will be expanded to search the whole PDB and correlate with other thiol-active site enzymes. Oxidised cysteines being incorrectly modelled as the reduced thiol, whilst likely physiologically accurate, means the built model would have a different electrostatic and steric environment which does not match the electron density. This has a compounding effect on any errors with deep learning and other modelling tools for accurate model building or automated drug discovery pipelines, which use this data for their training.

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## Modelling of radiation damage as a Gaussian process

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Damage to biological samples by ionising radiation is one of the factors fundamentally limiting the resolution achievable by cryo-EM [1]. As the effects of other factors are reduced, it becomes important to produce accurate models of radiation damage, as well as to develop computational methods for fitting them to cryo-EM datasets. Here, the low signal-to-noise ratio of cryo-EM micrographs presents a particular challenge to accurate modelling of the signal [2]. We have developed a physics-based model of protein radiation damage by cryo-EM, in which each atom is assumed to undergo a Brownian motion during imaging with possibly different motion parameters. The model has been used to derive the expected dependence of structure factors of the map on dose. We have fitted the model to several datasets by casting it as a Gaussian process. The resulting model is sufficiently flexible to account for the observed variation between structure factors yet can accurately extrapolate their values beyond the observations used in training.

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## Mechanistic Analyses of Radical Propagation in Protein CryoEM

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Radiolytic damage is the fundamental limit constraining structural interrogation of biological macromolecules by electron microscopy<sup>1</sup>. Although a wealth of literature precedent regarding strategies to mitigate the effects of radiolysis has been accumulated in crystallography, analogous work in single-particle cryoEM (SPA) remains underdeveloped<sup>2,3</sup>. Here we adopt a two-pronged approach to studying electron beam-induced radiolytic damage to organic and biomolecular samples. First, we use scanning nanobeam electron diffraction to analyze the isotropic propagation of delocalized radiolytic damage from a localized source in small-molecule organic crystals and protein crystals<sup>4</sup>. Second, inspired by these results, we employ dose-fractionated SPA reconstructions to analyze whether vitrifying samples in D<sub>2</sub>O rather than the conventional H<sub>2</sub>O leads to appreciable changes in global and site-specific damage.

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## Heavy-element damage seeding in proteins under XFEL illumination: How 10% NaCl can double the dose.

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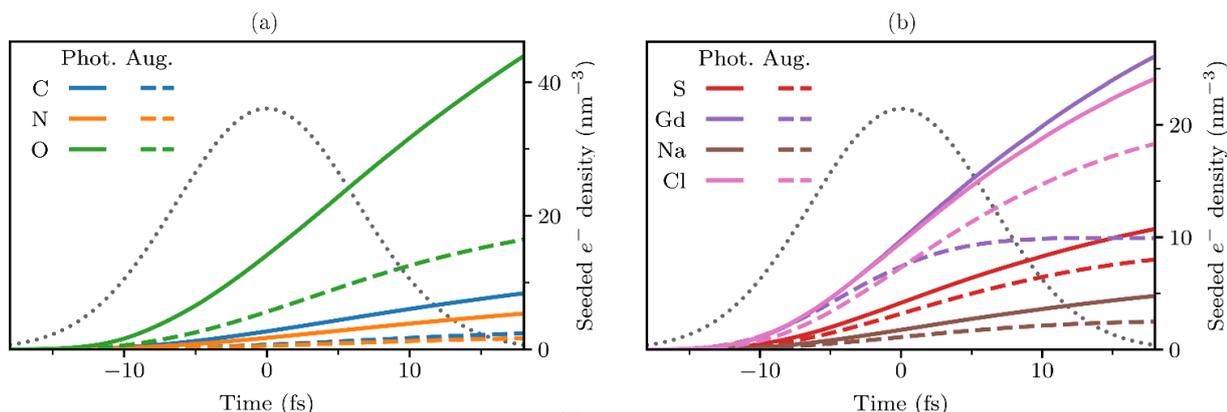
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Serial femtosecond X-ray crystallography (SFX) captures the structure and dynamics of biological macromolecules at high spatial and temporal resolutions. The ultrashort pulse produced by an X-ray free electron laser (XFEL) 'outruns' much of the radiation damage that impairs conventional crystallography. However, the rapid onset of 'electronic damage' due to ionization limits this benefit. Here, we distinguish the influence of different atomic species on the ionization of protein crystals by employing a plasma code that tracks the unbound electrons as a continuous energy distribution. The simulations show that trace quantities of heavy atoms ( $Z > 10$ ) contribute a substantial proportion of global radiation damage by rapidly seeding electron ionization cascades. In a typical protein crystal, sulfur atoms and solvated salts induce a substantial fraction of light-atom ionization. In further modeling of various targets, global ionization peaks at photon energies roughly 2 keV above inner-shell absorption edges, where sub-2 keV photoelectrons ejected from these shells initiate ionization cascades that are briefer than the XFEL pulse. These results indicate that relatively small quantities of heavy elements can substantially affect global radiation damage in XFEL experiments. We go on to examine how the composition of the solvent will affect refinement.



**Fig. 1.** Contribution of each element to secondary ionization in a lysozyme.Gd crystal ( $\text{H}_{2398}\text{C}_{615}\text{N}_{195}\text{O}_{887}\text{S}_{10}\text{Gd}_3\text{Na}_{19}\text{Cl}_{18}$ ) under a 15 fs Gaussian pulse with a fluence of  $1.75 \times 10^{12}$  7.1 keV  $\text{ph} \cdot \mu\text{m}^{-2}$ . Traces show the total free electron density of the electron ionization cascades seeded by the (a) light and (b) heavy elements in the target, due to photoionization (solid) or Auger decay (dashed). Such cascades drive global ionization.

## X-ray induced substrate decarboxylation in fatty acid photodecarboxylase observed by macromolecular and serial synchrotron crystallography

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Radiation damage (RD) is a significant problem in X-ray crystallography. High X-ray doses can cause two types of RD: global damage, which manifests itself in the reciprocal space and results in the degradation of a crystal's diffraction power, and specific damage, which is observed as changes in the electron density (real space)<sup>1</sup>. In macromolecules, specific damage includes, for example, the cleavage of disulfide bridges, the decarboxylation of acidic residues, and the reduction of metal centers or protein cofactors<sup>1</sup>. Fatty acid photodecarboxylase (FAP) is one of the few discovered photoenzymes, it is found in microalgae and catalyzes the decarboxylation of long- chain fatty acids into their corresponding hydrocarbon chains (alkane or alkene)<sup>2</sup>. FAP uses flavin adenine dinucleotide (FAD) as the light-capturing cofactor. The photocycle of decarboxylation in FAP involves a series of electron transfer steps from the FAD to the substrate's carboxyl group and *vice versa*, yielding the final photoproduct around 100 ns after photon absorption<sup>3</sup>. To date, the influence of high X-ray doses on protein substrates, particularly those located in the active site, has not been thoroughly investigated. In this study, we combined serial synchrotron X-ray crystallography (SSX) and macromolecular crystallography (MX), both performed at cryogenic temperature to analyze substrate behavior in wild-type FAP and three FAP mutants with differing catalytic efficiencies under increasing radiation doses. Doses ranged from 0.14 to 2.8 MGy and 0.83 to 8.3 MGy in the SSX and MX experiments, respectively. Our results show that the carboxyl group of the fatty acid substrate, when bound in the active site, is more radiation-sensitive than any protein residue. There appears to be no marked correlation between substrate radiation sensitivity and enzyme activity of mutants.

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## Exploring Specific Radiation Damage to Halogenated Ligands in Crystal Structures Collected at Cryogenic and Room Temperature

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Macromolecular X-ray crystallography is a powerful tool enabling modern structure-based drug design, where structures of protein-ligand complexes provide a basis for rational design decisions to improve the potency of ligands. However, like the protein itself, small molecules within protein crystals are subject to specific radiation damage (SRD) during the collection of X-ray diffraction data, but the effect of SRD on small molecule ligands has not yet been extensively described. This study is following on recently published work<sup>1</sup> investigating SRD to halogenated ligands in protein-ligand structures of the therapeutic cancer target B-cell lymphoma 6 protein (BCL6), which found significant cleavage of carbon-halogen (C-X) bonds during X-ray diffraction data collection. The present work is making use of an improved set of BCL6 ligands to gain further understanding of the role of the halogen type and substitution position on the susceptibility of the C-X bond to SRD. Additionally, diffraction data will not only be collected at cryogenic temperature, but also at room temperature at the VMXi beamline at Diamond Light Source to investigate the impact of data collection temperature on SRD to small molecule ligands.

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## Understanding the Mechanism and Health Consequences of Low-Dose Radiation at a Molecular Level

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The study of low-dose radiation effects remains a critical challenge in radiation biology, with implications for health and safety in various fields. The National Academies' 2022 report on „Leveraging Advances in Modern Science to Revitalize Low-Dose Radiation Research in the United States“ emphasizes the need for advanced technologies to precisely define the molecular and cellular consequences of low-dose exposures. These are many magnitudes below the doses used in X-ray crystallographic studies. Our research leverages structural biology from crystallography, AI-driven analysis, and experimental validation to address this gap. By analyzing the Protein Data Bank (PDB), we assess the impact of radiation damage at the atomic level, utilizing known signatures of radiation chemistry. We employ AI-based predictive models trained on high-dose data, a baseline of zero-dose neutron data, calculated dose and damage indicators, and the available literature, and we test and validate predictions against experimental observations. We aim to extrapolate radiation chemistry results to low-dose regimes. Additionally, we integrate small-angle X-ray scattering (SAXS) and neutron diffraction techniques to refine our understanding of radiation-induced structural changes and validate our predictions. Our approach combines machine learning with a digital twin framework, enhancing predictive capabilities and preserving decision-making insights for broader scientific applications. This research aims to identify proteins and pathways susceptible to low-dose radiation damage, elucidate underlying mechanisms, and develop mitigation strategies, contributing to both fundamental science and practical health considerations. We outline the initial stages of this research and our approach.

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