14th International Conference on Biology and Synchrotron Radiation

Sunday 11 June 2023 - Wednesday 14 June 2023 Lund

Book of Abstracts

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Program BSR 14

Sunday 11 June	
15:00 – 16:00	Registration (Location: Lunds standshallen)
16:00 - 17:30	Reception (Location: Lunds standshallen)

Monday 12 June	
09:00 - 09:30	Thomas Grant (University of Buffalo, USA) Ab initio electron density determination directly from solution scattering data, applications to drug discovery
09:30 - 10:00	Kartik Ayyer (MPI Hamburg, Germany) Machine learning to handle conformational heterogeneity in coherent imaging
10:00 - 10:30	Coffee
10:30 - 10:55	Eugene KRISSINEL (CCP4, UK), Structure Determination Online with CCP4 Cloud
10:55 - 11:20	Archana JADHAV, Archana (DLS, UK), A high-resolution correlative light and X-ray 3D cryo-imaging platform for cells and tissue at near-native physiological conditions
11:20 - 11:40	Yao-Chang LEE, (National Synchrotron Radiation Research Center, Taiwan) Medical application by using wax physisorption kinetics and Fourier transform infrared spectral imaging
11:40 - 12:00	Kunal SHARMA, (Lund University, Sweden) Multimodal characterization of heterotopic ossification during Achilles tendon healing in a rat animal model
12:00 - 13:00	Lunch
13:00 - 13:30	Connie Darmanin (La Trobe, Melbourne) Studies of Toll-like receptors using electron diffraction and FELS
13:30 - 14:00	Vadim Cherezov (University of Southern California, USA), Understanding GCPRs and their complexes
14:00 - 14:20	Jaehyun PARK, (Pohang Accelerator Laboratory, South Korea) Approaches to study biological systems at PAL-XFEL
14:20 - 14:40	Tobias KROJER, (MAX IV, Sweden) The FragMAX facility for structure-based drug discovery at MAX IV Laboratory
14:40- 15:00	Francesca SIRACUSA, (DTU, Denmark) Time-resolved phase contrast µCT measurements of nanoparticle transport in living plants.
15:00 - 15:15	Coffee
15:15 - 16:00	**Stephen Burley (Rutgers University, New Brunswick, USA) "Beyond the 50 years of the PDB"**
16:00 - 16:20	Peter GAAL, (TXproducts UG) WaveGate X-Ray Chopper for Synchrotron-Based Time-Resolved Serial Crystallography using the Hadamard Transform
16:20 - 18:00	Poster Session

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Tuesday 13 June	
09:00 - 09:30	Alexandra Pacureanu (ESRF, Grenoble, France) 3D synchrotron studies of the brain
09:30 - 10:00	Atsushi Momose (Tohoku University, Japan) Synchrotron Radiation Phase Imaging/Tomography Based on Grating Interferometry
10:00 - 10:30	Coffee
10:30 - 11:00	Marianne Liebi (Chalmers, Gothenburg, Sweden) SAXS tensor tomography in biomedical applications
11:00 - 11:30	Tim Salditt (Georg August University, Göttingen, Germany) Advances in 3-D imaging for bio-medical applications at modern synchrotrons
11:30 - 12:00	Colin NAVE, (Diamond Light Source, UK) Coherent Hard X-ray Bio-imaging at Diamond & Diamond II.
12:00 - 13:00	Lunch
13:00 - 13:30	Holger Stark (MAX Planck Institute, Göttingen, Germany) Structural insights into the spliceosome
13:30 - 14:00	Andrey Kovalevsky (ORNL, Oakridge, USA) Combined X-ray & neutron crystallography for drug design purposes
14:00 - 14:30	Britt Hedman (SLAC, Stanford, USA) Probing enzyme reaction mechanisms with XAS
14:30 - 15:00	Joanna CZAPLA-MASZTAFIAK, Complementary use of synchrotron and laboratory X-ray sources to study metal-based complexes in biological systems
15:00 - 15:30	Coffee
15:30 - 15:55	Oxana KLEMENTIEVA, (Lund University, Sweden) Correlative imaging to resolve molecular structures in individual cells
15:55 - 16:20	Andre CONCEICAO, (Deutsches Elektronen-Synchrotron DESY, Germany) Breast cancer metastasis progress based on the 3D collagen fibril orientation map
16:20 - 16:40	Margaux SCHMELTZ, (PSI, Switzerland) The human middle ear in motion: visualization and movement quantification using dynamic synchrotron-based X-ray microtomography
16:40 - 17:00	Irene RODRIGUEZ FERNANDEZ, (PSI, Switzerland) X-ray scattering methods to image bone healing around bio-resorbable implants RODRIGUEZ FERNANDEZ, Irene (Paul Scherrer Institut)
17:00 - 17:30	Travel to MAX IV
17.30 - end	MAX IV Visit

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Wednesday 14 June	
09:00 - 09:30	Aina Cohen (SLAC, Stanford, USA) Developments for macromolecular crystallography at the LCLS and SSRL
09:30 - 10:00	Helena Käck (Astra Zeneca, Mölndal, Sweden) Synchrotron and FEL studies for drug discovery: an industrial perspective
10:00 - 10:30	Coffee
10:30 – 10:55	Susan Nehzati (University of Saskatchewan, Sasketoon, Canada, MAX IV LU) Metals and human disease
10:55 - 11:20	Wojciech POTRZEBOWSKI (ESS, Sweden) Towards building and disseminating comprehensive publication guidelines for biomolecular small-angle scattering in an e-learning format
11:30 - 11:55	Isabella SILVA BARRETO (Lund University, Sweden) Micro- and nanostructure specific X-ray tomography to study collagen regeneration during tendon healing
11:55 - 12:45	Lunch
12:45 - 13:30	**Ian Wilson (Scripps Research Institute, La Jolla, USA) "Synchrotrons and Virus Research"**
13:30 - 14:00	Clement Blanchet (EMBL-Hamburg, Germany) Role of BioSAXS in the fight against coronavirus: from viral protein characterization to vaccine development.
14:00 - 14:30	Maximilian Ackermann (Johannes Gutenberg University Mainz, Germany) COVID19 – 3D imaging for deciphering the pathology of a global pandemic
14:30 - 15:00	Daniel ERIKSSON, (Australia) MX3: A new macromolecular crystallography beamline at the Australian Synchrotron
15:00 - 15:30	Coffee
15:30 - 16:00	Jill Trewella (University of Sydney, Sydney, Australia) Importance of validation in SAXS/SANS
16:00 - 16:25	Juan Sanchez Weatherby, (Diamond Light Source, UK) Routine room temperature protein structure determination in situ at Diamond beamline VMXi: current status and recent developments
16:25 - 16:50	Janina SPRENGER, (Deutsches Elektronen Synchroton) SARS-CoV-2 Methyltransferase ligand screening and peptide inhibitors
16:45 - 17:00	Closing remarks

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Abstracts oral presentations

COVID19 - 3D imaging for deciphering the pathology of a global pandemic

Maximilian Ackermann

Affiliation:

Institute of Clinical and Functional Anatomy, Johannes Gutenberg University Mainz (Germany)

Institute of Pathology, Helios University Clinics Wuppertal (Germany)

COVID-19 pneumonia revealed perivascular inflammation, an endothelial injury, microangiopathy, and an aberrant blood vessel neoformation by intussusceptive angiogenesis. The pathomechanism by which SARS-CoV2 causes the fatal trajectory of pulmonary pathology COVID-19 still remains vague. We studied a total of 85 lungs (COVID autopsy ; influenza A; ILD explants; healthy controls) using the highest resolution Synchrotron radiation-based hierarchical phase-contrast tomography, scanning electron microscopy of microvascular corrosion casts, IHC, MALDI-TOF, and analysis of mRNA expression and biological pathways. Plasma samples from all disease groups were used for liquid biomarker determination using ELISA. The observed mosaic appearance of COVID-19 in conventional lung imaging resulted from microvascular occlusion and secondary lobular ischemia. The length of hospitalization was associated with increased intussusceptive angiogenesis. This was associated with enhanced angiogenic, and fibrotic gene expression demonstrated by molecular profiling and metabolomics. Plasma analysis confirmed distinct fibrosis biomarkers (TSP2, GDF15, IGFBP7, Pro-C3) that predicted the fatal trajectory in COVID-19. Pulmonary severe COVID-19 is a consequence of secondary lobular microischemia and fibrotic remodelling, resulting in a distinctive form of fibrotic interstitial lung disease that contributes to long-COVID

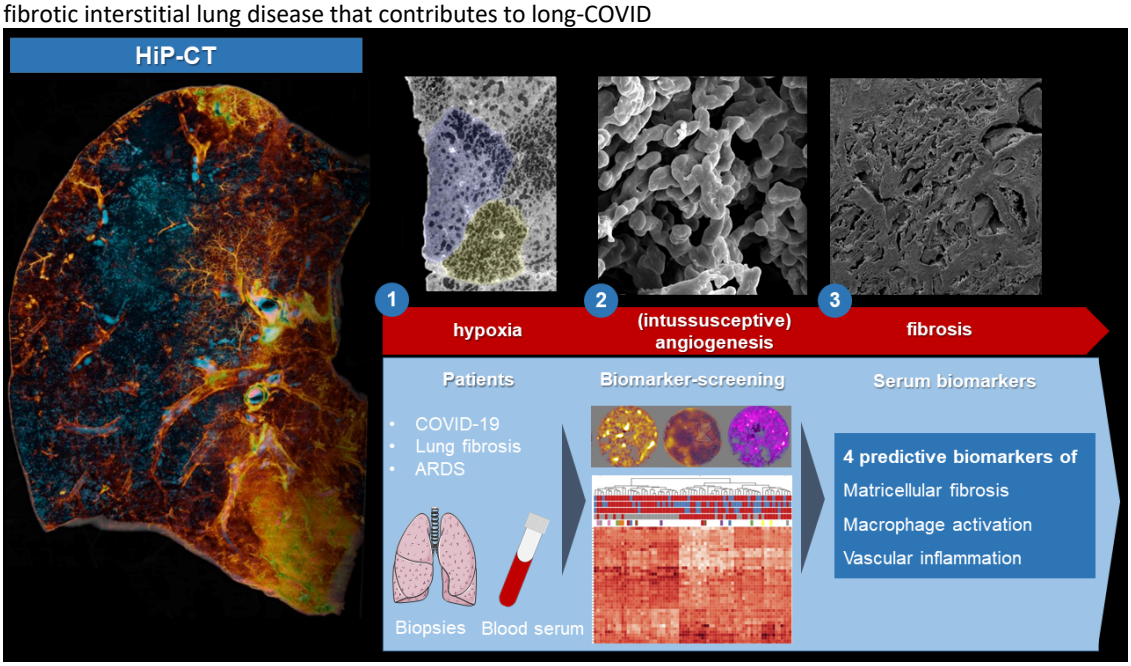

Figure 1: With the help of synchrotron-based hierarchical phase-contrast tomography (HiP-CT), a mosaic-like hypoxic undersupply of the smallest functional unit of the in severe COVID-19 lungs, the lung lobules, could be shown for the first time. This hypoxia and vascular damage caused by the SARS-CoV-2 virus leads to excessive formation of new blood vessels, so-called intussusceptive angiogenesis, which leads to scarring and fibrosis of the lung tissue in a very short time via inflammatory processes. In order to identify potential therapeutic targets or progression biomarkers, blood serum and biopsy tissue from patients with different COVID-19 progressions, pulmonary fibrosis (IPF) and acute lung injury (ARDS) were analysed and validated in a broad screening approach using proteomics and metabolomics. Three matricellular biomarkers and one macrophage-derived biomarker were identified as predictive blood-biomarkers that predict the progression of the scarring process.

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Breast cancer metastasis progress based on the 3D collagen fibril orientation map

Presenter: CONCEICAO, Andre (Deutsches Elektronen-Synchrotron DESY)

Co-authors: Dr HAAS, Sylvio (DESY); Prof. MÜLLER, Volkmar (Universitätsklinikum Hamburg-Ep- pendorf); Dr BURANDT, Eike (Universitätsklinikum Hamburg-Eppendorf); Dr MOHME, Malte (Uni- versitätsklinikum Hamburg-Eppendorf)

Content

Breast cancer is the leading cause of cancer death in women worldwide [1]. In these patients, more than 90% of breast cancer-related deaths are caused not by the primary tumor, but by their metastases at distant sites. Metastasis is an extraordinarily complex process, entailing tumor cells to acquire a set of features that allow them to develop new foci of the disease. Systemic changes in the microenvironment between the cancer cells and the host stroma play an important role in sup- porting the growth and progression of the tumor by degrading, re-depositing, cross-linking and stiffening collagen fibrils. Although the knowledge of breast carcinogenesis is being progressively elucidated with 2D cell-culture experiments, they are not able to reproduce the real physiological pattern of the tumor microenvironment where the surrounding cells are equally as important as the tumor cell itself. Small-angle X-ray scattering has been successfully exploited to observe the organization of collagen fibrils in breast tissues [3]. To probe the remodeling of collagen fibrils in breast tumors in a volume-resolved way, it was exploited the potential of Small-angle X-ray Scattering Tensor Tomography (SASTT) in providing six-dimension images. Therefore, this study aimed to provide the basis to help in deciphering the mystery organ-specific metastasis.

The SASTT experiment was carried out at the SAXSMAT P62 beamline at the PETRA III storage ring in Hamburg, Germany. The SAXSMAT beamline has dedicated instrumentation to perform such an experiment. It used a 12.4 keV monochromatic beam focused to have 20 x 20 µm2 at the sample position to scan horizontally and vertically at several rotations and tilt angles with 50 ms exposure time. The region of interest of the freeze-dried breast tumor samples was selected and cut in a cylindrical shape with 1mm diameter and 1.5 mm height and placed on top of the tomo-graphic stage system. A two-dimensional single-photon counting detector, Eiger2-9M ®DECTRIS, positioned at 3854 mm from the sample was used to record the photons scattered at small-angle, covering a q-range between 0.06 nm-1 and 1.95 nm-1. An in-house python-based pipeline data processing and reconstruction was used to average each of the 2,034,900 SAXS patterns and fur ther reconstruct it.

A higher degree of content and orientation of the collagen fibrils was observed in the SASTT im- ages at the tumor region previously indicated by an expert breast pathologist as a metastatic area. The orientation of the collagen fibrils to the tumor boundary can be an important sign of how the invasion of tumor cells into the stroma and migration toward the blood or lymphatic stream to a secondary organ is triggered.

Synchrotron Radiation Phase Imaging/Tomography Based on Grating Interferometry

Atsushi Momose
Institute of Multidisciplinary Research for Advanced Materials, Tohoku University
2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan

X-ray grating interferometry, such as Talbot interferometry, has been used as one of major techniques for phase imaging and phase tomography. The limiting factor of its achievable spatial resolution is the period of the gratings consisting of a grating interferometer, but it can be overcome by combining with the optics of X-ray microscope [1]. We have been studying several configurations for this purpose, including a super-resolution approach. A direct combination of a Talbot interferometer and a FZP-based full-field X-ray microscope [2, 3] is steadily operated at SPring-8, Japan. It was designed considering the compatibility between a microscopic spatial resolution and a wide field of view rather than aiming at a frontier spatial resolution, and has been applied to bone samples [4,5]. I will introduce the latest development status with some application cases.

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Role of BioSAXS in the fight against coronavirus: from viral protein characterization to vaccine development.

Presenter: BLANCHET, Clement (EMBL Hamburg)

Content

Biological small angle scattering is a key method in biophysics and structural biology. It pro- vides information on the size, shape, flexibility, and oligomeric composition of biological macro- molecules. SAXS measure the sample directly in solution, in a quasi-native environment and is complementary to high resolution methods such as X-ray crystallography and Cryo-EM. In particu- lar, structural changes triggered by modifications of the sample conditions (change in temperature, pH, salt or ligand concentration) can be monitored in screening or time-resolved experiments.

BioSAXS contributed to the description of the SARS-CoV-2 virus and to the development of vac- cine and potential drugs. Its role in the fight against coronavirus will be illustrated with several projects carried out on the P12 beamline of EMBL Hamburg. SAXS was employed, for example, to characterize the topology of proteins involved in RNA replication for different coronavirus1. SAXS helped characterize the spike protein and its interaction with synthetic nanobodies that could neu- tralize the virus2. Beyond the study of the viral components, SAXS is also used to characterize and design the lipid nanoparticules that carries messenger RNA in new generation vaccine3,4,5.

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Probing Enzyme Reaction Mechanisms with XAS

Presenting Author Britt Hedman

bhedman@stanford.edu

Professor of Photon Science, Stanford University
Science Director, Stanford Synchrotron Radiation Lightsource

1 Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Stanford University, Stanford, CA, USA

Metal ions have key roles in biological structure and function - from being active sites of many enzymes to shuttling electrons in key metabolic pathways, having roles in signaling pathways and being key elements of cancer chemotherapies and disease-related biological malfunctions. X-ray absorption spectroscopy (XAS) provides a unique tool to study biological structure in that it provides, at the molecular level, element-specific local structural information, highly complementary to that from macromolecular crystallography. It can furthermore be applied to most physical forms of biological systems, such as as-isolated dilute metalloenzyme solutions, single crystals, cells, and tissues, for which spatially resolved information about metal distribution and speciation over different length scales and resolution has become increasingly useful. The XAS near-edge structure is sensitive to the electronic structure and geometric arrangement around an absorbing atom site, while the extended x-ray absorption fine structure energy region provides information on in particular the first-shell coordination sphere, and in some cases also outer coordination, in particular if multiple-scattering paths are present and can be determined. X-ray emission (XES) and resonant inelastic x-ray scattering techniques (RIXS) provide enhanced information on electronic structure, reaching into the time domain. This talk will describe some experimental approaches and a few biological applications.

Medical application by using wax physisorption kinetics and Fourier transform infrared spectral imaging

Presenter: Dr LEE, Yao-Chang (National Synchrotron Radiation Research Center) **Co-author:** Mrs HUANG, Pei-Yu (National Synchrotron Radiation Research Center)

Content

Wax physisorption kinetics with FTIR (WPK-FTIR) imaging provides a glycol-histopathological imaging analysis for differentiating the abnormal glycosylation within medical tissue sections and cell lines, utilizing n-alkanes (n-CnH2n+2) with varied carbon number (CN) from 20 to 34 and beeswax as wax adsorbents for targeting similar longitudinal length of glycans of glycoconjugates anchoring in the medical sample surface based on chemical similarity principle. WPK-FTIR is an in-situ non-destructive method of examining tissue sections for cancer screening and prognosis prediction for chronic kidney disease by profiling aberrant glycans covalently attached to both glycoconjugates anchored in tissue sections. Currently, WPK-FTIR can screen for ten kinds of cancer disease, including colon cancer, breast cancer, ovary cancer, cervical cancer, oral cavity cancer, gastric cancer, skin cancer, prostate cancer, intestinal neuroendocrine tumour, and brain cancer.

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3d virtual histology with synchrotron radiation: from optics and phase retrieval to multi-scale and high throughput studies

Tim Salditt

Institute for X-ray Physics, University of Göttingen, Göttingen, Germany tsaldit@gwdg.de

In order to unravel physiological and pathological mechanisms, structure and processes have to be visualized over a wide range of scales. High brilliance synchrotron radiation makes it possible to image larger tissue volumes at cellular and sub-cellular resolution [1]. This meets an increasing need, as biological and biomedical research increasingly shifts focus from cell culture to tissues, to model organisms and to organoids.

In order to visualise and to quantify the cytoarchitecture in 3D, even deep in the tissue or organ, we use multi- scale phase-contrast imaging based on coherent wavefield propagation in combination with advanced phase retrieval [2-4]. This enables a new form of fully digital 3D virtual histology [5]. We have implemented the method both for parallel beam and holographic projections recordings with nano-focusing optics. Since the workflow is non-destructive and fully compatible with standard clinical pathology, we can perform correlative histology studies. In this talk we discuss the current capabilities and further developments of optics, phase retrieval, and reconstruction in view of resolution, dose, throughput, and how to find the 'needle in the haystack'? What limits apply to 'zooming-in', and which probe, optic, algorithms are best suited to exploit the opportunities of 4th generation synchrotron sources.

We give examples for applications in three-dimensional virtual histology and patho-histology, offering new insights into the cytoarchitecture of human organs [5-7], from lung and heart to brain, and the respective pathological alterations. And finally we discuss: *how to exploit all this data*?

Keywords – Holo-Tomography, Phase retrieval, Multi-scale imaging, Biological Imaging

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3D virtual histopathology of cardiac tissue from Covid-19 patients based on phase-contrast X-ray tomography eLife (2021), 10:e71359

Structure Determination Online with CCP4 Cloud

Primary authors: Dr KRISSINEL, Eugene (CCP4); Dr LEBEDEV, Andrey (CCP4); Ms FANDO, Maria (CCP4); Dr BALLARD, Charles (CCP4); Dr USKI, Ville (CCP4); Dr KEEGAN, Ronan (CCP4)

Presenter: Dr KRISSINEL, Eugene (CCP4)

Content

CCP4 Cloud is a front-end for the CCP4 Software Suite, which represents a new approach to de- veloping, organising and maintaining crystallographic projects and running CCP4 programs. It exploits the paradigm of remote computing, when all projects and data are kept online and tasks are executed on remote servers. CCP4 Cloud is designed for deployment at scientific centres and data-producing facilities (synchrotron beamlines). The system complements remotely driven, on- line experiments with online structure solution, starting from image processing to deposition in the PDB.

Obvious benefits of a fully online approach to structure determination are in the optimal use of re- sources, both experimental and computational, and systemic approach to data management, which includes retention of structure solution projects, the usually missing link between experimental data and their interpretation in form of solved structures.

CCP4 Cloud includes tasks for all stages of computational structure determination and provides integrated access to relevant web-resources (PDB, UniProt, AlphaFold, ESM etc.) and 3rd-party software (e.g., Buster from Global Phasing and PDB-REDO from NKI, Amsterdam). A set of au- tomated tools are provided for image processing, phasing, model building, refinement, validation and preparation for deposition in the PDB. As a unique feature, CCP4 Cloud offers automatic development of structure solution projects, which can deliver a full solution, and allow for user intervention when a good-quality solution is not achieved. CCP4 Cloud projects can be initiated online in a web-browser or via background push of data from point of collection.

CCP4 Cloud facilitates teamwork by providing a shared access to structure solution projects, when several researchers can work simultaneously in real time on the same project. The projects can be also exchanged as zip files and stored locally in personal archives. Alternatively, projects can be archived in CCP4 Cloud and made accessible via stable web-links similar to DOI, a feature that can be used at publishing, refereeing, education and exchange within a wider collaboration.

CCP4 maintains a publicly available instance of CCP4 Cloud at https://cloud.ccp4.ac.uk, and sev- eral private instances have been deployed at other academic and industrial sites. CCP4 Cloud is included in standard CCP4 distribution and runs out-of-the-box on the user's machine. CCP4 encourages wider use of CCP4 Cloud, especially at data-producing facilities, in hope that it will grossly improve and streamline user experience in MX structure determination, data and project management.

The human middle ear in motion: visualization and movement quantification using dynamic synchrotron-based X-ray microtomography

Presenter: Dr SCHMELTZ, Margaux (PSI)

Co-authors: IVANOVIC, Aleksandra (Paul Scherrer Institut, Inselspital - University Hospital Bern); Dr SCHLEPÜTZ, Christian (Paul Scherrer Institute); Dr WIMMER, Wilhelm (Technical University of Mu- nich); Dr REMENSCHNEIDER, Aaron (Harvard Medical School, UMass Chan Medical School); Prof. CAVERSACCIO, Marco (Inselspital Bern University Hospital); Prof. STAMPANONI, Marco (Paul Scher- rer Institute, ETHZ); Dr BONNIN, Anne (Paul Scherrer Institute); Dr ANSCHUETZ, Lukas (Inselspital Bern University Hospital)

Content

Characterizing the movement of the middle ear ossicles during sound transmission is of prime interest in clinical research. However, the small size, the location within the temporal bone, and the tiny movements of the three ossicles of the human middle ear make this type of measurements extremely challenging.

In this work, we use dynamic synchrotron-based X-ray phase-contrast micro-tomography to visu- alize the 3D motions of the intact human tympanic membrane and ossicular chain under acoustic stimulation. An in-house built fast read-out system coupled with a specific post-gating algorithm provided the temporal capability to resolve periodic micromotions up to 750 Hz.

A high-throughput pipeline optimized for the large tomographic datasets enabled to quantitatively describe the rigid-body motion of the ossicles from seven fresh-frozen healthy human ears stimu- lated at various acoustic frequencies and intensities. The displacement of several points of interest within the ossicular chain were analyzed and compared to the motions currently found in literature.

Approaches to study biological systems at PAL-XFEL

Presenter: Dr PARK, Jaehyun (Pohang Accelerator Laboratory)

Co-authors: Mr PARK, Sehan (Pohang Accelerator Laboratory); Dr LEE, Sang Jae (Pohang Accel- erator Laboratory); Dr KIM, Jangwoo (Pohang Accelerator Laboratory); Dr KIM, Minseok (Pohang Accelerator Laboratory); Dr EOM, Intae (Pohang Accelerator Laboratory)

Content

PAL-XFEL operates user beamtime for various scientific studies related to physics, chemistry, and biology since 2017. It especially provides XFEL pulses in a very high quality for the timing jit- ter and stability [1, 2]. To unveil the macromolecular structures at room temperature and nearly atomic resolution, we run the serial femtosecond crystallography (SFX) science program at the NCI experimental station that delivers high-density photons with K-B mirror optics. We have devel- oped and facilitated various sample delivery techniques to deal with a small sample consumption and complex configuration for the time-resolved studies [3, 4]. According to the sample characteristics and purpose of the experiment, we provide specific instruments to deliver micro-crystals. The crystal delivery techniques can be categorized into liquid flow and fixed target systems.

For the liquid flow scheme, the carrier matrix delivery (CMD) injector is used for the highly viscous media requiring high pressure to put the crystals to the X-ray interaction point. On the other hand, micro-liter volume (MLV) syringe injector can be applied to the relatively lower viscous samples. The MLV syringe injector is also available to perform time-resolved SFX experiments accompanied with multiple optical pump lasers simultaneously. On the other hand, the fixed target (FT) system has a good advantage of simple and stable operation during the crystal delivery process. We have developed two kinds of FT systems, 2D and 1D configurations [5, 6]. The 2D FT system is composed of a mesh and two thin films to keep the hydrated condition and reduce crystal falling due to the gravity during the scanning. To pursue a decrease in sample consumption, the 1D FT system has been developed, which utilizes microtubing to contain micro-crystal slurries. It also has a strong point to handle the samples while maintaining humid environment. In addition, we are under development for other instruments to provide more opportunities to the users regarding time-resolved studies based on domestic and international collaborations.

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[2] I. Nam *et al.*, *Nat. Photonics* **15**, 435 (2021)
[3] J. Park *et al.*, *J. Sync. Rad.* **25**, 323 (2018)
[4] I. Eom *et al.*, *Appl. Sci.* **12**, 1010 (2022)
[5] D. Lee *et al.*, *Sci. Rep.* **9**, 6971 (2019)
[6] D. Lee *et al.*, *J. Appl. Cryst.* **53**, 477 (2020)

Towards building and disseminating comprehensive publication guidelines for biomolecular small-angle scattering in an e-learning format

Presenter: POTRZEBOWSKI, Wojciech (Data Managment and Software Centre, European Spallation Source, ERIC)
Primary authors: POTRZEBOWSKI, Wojciech (Data Managment and Software Centre, European Spallation Source, ERIC); Ms ALIYAH, Kinanti (Paul Scherrer Institut)

Content

Biological Small Angle Scattering is a technique that is widely used to characterize samples of biomolecules in solution. Despite thousands of data sets generated every year, only a fraction of them is published, and even a smaller fraction is presented in a way that results can be reproduced, and unbiased conclusions can be drawn. A reporting guideline, specifically targeted for structural modelling of small-angle scattering data, was published in 2017 to facilitate the reviewer's and the reader's independent assessment of the data quality and its interpretation. [1] More recently, an updated template was published for the general biomolecular structural modelling SAS experiment and an additional specific SAS-contrast variation template. [2] A primary motivation for the endeavour is to launch a community consensus on what should be in a publication, together with standardized data quality assurance and the employment of agreed-upon model validation methods. The standardized measure also, in turn, instils confidence in the results, concerning a broader structural biology community.

By leveraging the already laid-out foundation from the guideline templates, we initiated an effort to distil the dense information from the papers into a more systematic and engaging format with the e-learning platform (https://e-learning.pan-training.eu/). The e-learning course would be a beneficial platform to disseminate the guidelines more interactively. Furthermore, the updated templates are available to be downloaded from the platform, divided into relevant sections of data pipeline/measurement type, and populated as the SAS measurement goes. This eases the rather lengthy effort if the table needs to be populated all at once near a measurement's publication sub- mission. Therefore, we invite experienced biomolecular SAS experts to contribute further to the platform. But, more importantly, the students/users beginning their journey using biomolecular SAS as a tool in the structural investigation are invited to take advantage of the platform. The presentation will detail the content of the e-learning platform and its most updated features, such as interactive quizzes and useful up-to-date links for the tools frequently used in biomolecular SAS, as well as ways to contribute.

Bone density distribution in the human auditory ossicles: A synchrotron-based phase-contrast microtomography study of the human middle ear

Presenter: IVANOVIC, Aleksandra (Paul Scherrer Institut, Department of Otorhinolaryn- gology, Head and Neck Surgery, Inselspital, Bern University Hospital, Hearing Research Laboratory, ARTORG Center for Biomedical Engineering Research, University of Bern)

Co-authors: SCHALBETTER, Fabian (Department of Otorhinolaryngology, Head and Neck Surgery, Inselspital, Bern University Hospital); Dr SCHMELTZ, Margaux (PSI); Dr BONNIN, Anne (Paul Scherrer Institute); Prof. ANSCHUETZ, Lukas (Department of Otorhinolaryngology, Head and Neck Surgery, Inselspital, Bern University Hospital, Hearing Research Laboratory, ARTORG Center for Biomedical En- gineering Research, University of Bern)

Content

The human middle ear hosts the three auditory ossicles, which ensure sound transmission from the environment to the inner ear through impedance matching. Unlike for long bones, the ossification of the human hearing bones is completed shortly after birth, and only little ossicular remodeling of the bone has been observed afterwards[1-4]. Nevertheless, studies have shown that the over- all mineralization is much higher than for long bones[2]. Accordingly, the mineralization of the ossicles is crucial for the proper functioning of sound transmission[1-5]. Previous literature also hypothesizes that, similar to long bone, there is a different degree of bone remodeling, and hence bone density, along the ossicles due to the different loads in this tiny biomechanical system[3,6]. But, little is known about the bone density distribution along the human ossicular chain and within each ossicle itself.

In this study, we used synchrotron-based X-ray phase-contrast microtomography to scan three fresh-frozen human middle ears at the TOMCAT beamline (X02DA) of the Swiss Light Source (Paul Scherrer Institut) at a pixel size of 2.75µm.

We reconstructed the datasets using the phase retrieval method of Paganin[7] to obtain the best possible contrast for defining the bone density distribution. However, to calculate the volume and porosity of each ossicle, we used the reconstructions in absorption only to ensure the best reso- lution. This gained knowledge will help the ENT community to understand the pathophysiology of the middle ear ossicles better and therefore improve the surgical outcome of autologous graft placement.

[1] Delsmann et al., "Prevention of Hypomineralization in auditory ossicles of Vitamin D receptor deficit mice," Frontiers in Endocrinology (2022)

[2] Kuroda et al., "Hypermineralization of hearing-related bones by a specific osteoblast subtype," JBMR (2021)

[3] Morris et al., "Bone Mineral Density of Human Ear Ossicles," Clinical Anatomy (2018)

[4] Rolvien et al., "Early bone tissue aging in human auditory ossicles is accompanied by excessive hypermineralization, osteocyte death and micropetrosis", Sci Rep 8, 1920 (2018)

[5] Kanzaki et al., "Impaired Vibration of Auditory Ossicles in Osteopetrotic Mice," The American Journal of Pathology (2011)

[6] Anschuetz L. et al., "Synchrotron Radiation Imaging Revealing the Sub-micron Structure of the Auditory Ossicles", Hearing Research (2019)

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WaveGate X-Ray Chopper for Synchrotron-Based Time-Resolved Serial Crystallography using the Hadamard Transform

Presenter: GAAL, Peter (TXproducts UG)

Primary authors: Mr SCHMIDT, Daniel (TXproducts UG); Dr VON STETTEN, David (EMBL Hamburg); Dr AGTHE, Michael (EMBL Hamburg); Dr YORKE, Briony A (The University of Leeds); Dr BEDDARD, Godfrey S. (University of Edinburgh); Prof. PEARSON, Arwen R. (6) Hamburg Centre for Ultrafast Imaging & Institut for Nanostructure and Solid State Physics); GAAL, Peter (TXproducts UG)

Content

We present a new method for tailoring the time-structure of hard X-ray synchrotron beams for time-resolved serial crystallography. The central element of this method is a variable solid-state X- ray chopper called the WaveGate. It can span arbitrary temporal gates with durations of 100 ns to milliseconds and longer. In this project we employ the WaveGate to generate complex sequences of x-ray probe pulses based on the Hadamard transform, which allow a faster time-resolution than would be expected with fast pump-probe experiments for flux limited sources.

We will present a full characterization of the WaveGate chopper and demonstrate the feasibility of synchrotron-based time-resolved measurements using the Hadamard transform.

Multimodal characterization of heterotopic ossification during Achilles tendon healing in a rat animal model

Presenter: Mr SHARMA, Kunal (Lund University, Sweden)

Primary authors: Mr SHARMA, Kunal (Lund University, Sweden); Ms SILVA BARRETO, Is- abella (Lund University, Sweden); Dr DEJEA, Hector (Lund University, Sweden. MAX IV Laboratory, Sweden); Dr HAMMERMAN, Malin (Linköping University, Sweden. Lund University, Sweden); Dr GERAKI, Tina (Diamond Light Source, Oxfordshire, United Kingdom); Dr ELIASSON, Pernilla (Depart- ment of Orthopaedics, Sahlgrenska University Hospital, Gothenburg, Sweden); Dr PIERANTONI, Maria (Lund University, Sweden); Prof. ISAKSSON, Hanna (Lund University, Sweden)

Content

Introduction: Clinical case studies of healing Achilles' tendons identified localized mineral de- positions radiographically, described as 'heterotopic ossifications' (HO), or 'bone-like' tissue. The presence of HO has been associated with increased risk of re-ruptures and pain 1. The origin of HO in healing tendons is not yet fully understood. This study characterizes elementally and structurally the HO in healing tendons at the nanoscale.

Methods: Rat Achilles' tendons of Sprague-Dawley rats (10-14 weeks old) were transected and al- lowed to heal [2] for 3- and 12-weeks (N=3, N=2, respectively). The healing tendons were fixed, em- bedded in PMMA, and sectioned to 5□m. Simultaneous micro-X-ray fluorescence (XRF) and diffrac- tion (XRD) were conducted at I-18, Diamond Light Source (12.0keV, 2 x 2µm2 step size). Structural properties of HO were calculated by the Scherrer equation to yield the L- and W-parameters cor- relating to crystallite size along the c-axis, and the ab-plane respectively [3].

Results: Calcium (Ca) content is increasing between time points. Higher localized amounts of Zinc (Zn) and Iron (Fe) were present at the edges of HO preceding both higher Ca, and crystal- lite dimensions (L- and W-parameters). Zn was also observed around larger voids at 12-weeks, comparable visually to calcaneal bone, indicating ongoing remodelling.

Discussion: Spatial distribution of Zn is consistent with the mineralization process seen in em- bryonic mice [4]. Our results provide insight into key performers in the process of tendon tissue mineralization, and aid in the understanding of HO formation during the Achilles tendon healing process.

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Complementary use of synchrotron and laboratory X-ray sources to study metal-based complexes in biological systems

Presenter: CZAPLA-MASZTAFIAK, Joanna (Institute of Nuclear Physics PAN)

Co-authors: Ms WIKTORIA, Stańczyk (Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland); Dr WOJCIECH, Błachucki (Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland); Mr RAFAŁ, Fanselow (Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland); Prof. JACINTO, Sa (Department of Chemistry, Uppsala University, Uppsala, Swe- den); Prof. JAKUB, Szlachetko (SOLARIS National Synchrotron Radiation Centre, Jagiellonian Univer- sity, Kraków, Poland); Prof. WOJCIECH, Kwiatek (Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland).

Content

Many metal complexes are widely known for their activity in biological systems. In addition to the compounds occurring naturally in the human body, metal ions are also required for their role as pharmaceuticals as well as diagnostic agents. Due to the increasing number of cancer cases in the world, research into new anti-tumor drugs containing metal ions is particularly important. Therefore, our research is focused on interaction of metal-complexes with cell constituents and their potential anti-tumor activity. Through the advance of synchrotron methods, as well as the development of a laboratory setup for XAS and XES measurements, we have created complemen- tary procedures enabling chemical characterization of metal complexes of biological importance. Herein I will present our results of studies of platinum and copper complexes utilizing X-ray spec- troscopy with laboratory and synchrotron X-ray sources. Proposed methodology of research can be used to obtain information about chemical structure of studied metal complexes, hydrolysis as well as interaction mechanism with other biomolecules. This approach was successfully imple- mented in the studies of cisplatin 1, novel platinum drugs [2,3] and copper complexes with phenan- throline [4]. Moreover, the possibility of application of novel methods, such as chronoscopy, which can be used on pulse X-ray sources, and give us the information about dynamics of the studied interactions, will be presented.

We acknowledge National Science Centre, Poland (NCN) for partial support under grants no. 2016/21/D/ST4/00378 and 2017/27/B/ST2/01890.

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Micro- and nanostructure specific X-ray tomography to study collagen regeneration during tendon healing

Presenter: SILVA BARRETO, Isabella (Lund University, Sweden)

Primary authors: SILVA BARRETO, Isabella (Lund University, Sweden); PIERANTONI, Maria (Lund University, Sweden); NIELSEN, Leonard (Chalmers University of Technology, Sweden); HAM- MERMAN, Malin (Lund University, Sweden; Linköping University, Sweden); DIAZ, Ana (Paul Scherrer Institut, Switzerland); NOVAK, Vladimir (Paul Scherrer Institut, Switzerland); ELIASSON, Pernilla (Linköping University, Sweden; Sahlgrenska University Hospital, Sweden); LIEBI, Marianne (Chalmers University of Technology, Sweden; Paul Scherrer Institut, Switzerland; Ecole Polytechnique fédérale de Lausanne, Switzerland); ISAKSSON, Hanna (Lund University, Sweden)

Content

Introduction: The recovery of the collagen structure following Achilles tendon rupture is poor, re- sulting in high risk for re-ruptures (1). The loading environment affects the mechanical properties of healing tendons but knowledge regarding how it affects regeneration of the tendon structure is still limited. This study characterizes the effect of reduced in vivo loading on the regenerating 3D micro- and nanoscale tissue structure.

Methods: Rat Achilles tendons were characterized during early healing (1 and 3 weeks) by com- paring full cage activity (FL) with immobilization (UL) (N=1/group) (2). The healing tendons were fixed in formalin and measurements conducted in the central part of the callus. The 3D organiza- tion of microscale collagen fibers was visualized by phase-contrast microtomography (PC-µCT) at TOMCAT beamline, PSI (15 keV, 1.63 µm pixel size), and the 3D organization of nanoscale colla- gen fibrils by small-angle X-ray scattering tensor tomography (SASTT) (3) at cSAXS beamline, PSI (12.4 keV, 150 µm beam size).

Results: Unloading during early tendon healing led to generally less collagenous material being formed and a larger presence of adipose tissue (Fig 1.A). The newly formed fibrils and fibers in unloaded tendons were less packed, more disorganized, and less longitudinally oriented along the main axis of the tendon (Fig 1.B).

Discussion: The structural effects due to unloading may explain the reported impaired mechani- cal competence of the tissue following immobilization during tendon healing (1). Ultimately, this study provides proof of concept of SASTT to study tendon tissue and its potential to investigate other soft collagenous tissues.

(1) Notermans et al., European Cell and Materials, 2021

(2) Hammerman et al., PLoS One, 2018

(3) Liebi et al., Nature, 2015

![Fig 1. A) Microscale organization obtained by PC-µCT (arrowhead = adipose tissue) and B) nanoscale organization obtained by SASTT (number of glyphs = fibril amount, glyph direction = fibril orientation, glyph colour = degree of fibril orientation) in a 3 weeks unloaded (UL) and fully loaded (FL) healing tendon. 1

Time-resolved phase contrast µCT measurements of nanoparticle transport in living plants.

Presenter: SIRACUSA, Francesca (DTU)

Co-authors: ØSTERGAARD, Emil Visby (Technical university of Denmark); MOKSO, Rajmund (Max IV Laboratory, Lund University)

Content

The transition to green agriculture faces several challenges. One major issue is the inefficient use of soil-based, conventional fertilizers. A solution to increase both the efficiency and flexibility of fertilization is to bypass the whole soil system and develop the concept of foliar fertilization. With the development of nanotechnology, new avenues have opened with the potential to truly revolu- tionize this concept. Recent observations suggest that mineral nutrients (P, Mn, and Zn) delivered as nanoparticles (NPs) can enter the plant through the leaves and be delivered to the target location inside the plant 1,[2]. However, several instances of the NPs pathways rely on hypotheses rather than experimental evidence. For example, the role of stomata is significant, yet remains poorly understood.

To shed light on how NP transports inside plants, we combine X-ray imaging, spectroscopy and scattering methods at synchrotrons and laboratory micro-tomography in the 3D imaging center at DTU. We will show results obtained with freeze-dried and living plants. In the latter case, perform longitudinal studies of different particle types (MnO, ZnO filled Mesoporous silica nano shells and hydroxyapatite) applied onto the leaves of Soy and barley plants. Synchrotron nano-tomography [Fig 1a shows Spring-8 data] reveals the 3D cellular ultrastructure and NP aggregates, while with SAXS (Small angle X ray scattering) and XRF we can identify non-aggregated nanoparticles in the plant tissue. The accumulated high density particles indicated by arrows in Fig 1c are perhaps the first direct visualization of foliar applied NP translocation in the vascular tissue of plants. We are currently performing validation studies for this claim.

Careful X-ray dose optimization allowed us to capture the 3D microstructure of living plants with applied NPs at multiple time frames. With the first commercial propagation based phase con- trast tomograph installed at 3D imaging center at DTU we are investigating the NP transport in plants for up to 6 days from foliar application, while with synchrotron imaging we focus on sub- micrometer resolution 3D representation of cellular plant structures involved in NP transport.

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[2] Burkhardt, J. (2010). Hygroscopic particles on leaves: nutrients or desiccants? Ecol. Monogr. 80, 369-399

Combined X-ray and neutron crystallography for drug design purposes

Presenter: KOVALEVSKY, Andrey (Oak Ridge National Lab)

Content

COVID-19, caused by SARS-CoV-2, remains a global health threat even with available vaccines and therapeutic options. The viral main protease (Mpro) is indispensable for the virus replication and thus is an important target for small-molecule antivirals. Computer-assisted and structure- based drug design strategies rely on atomic scale understanding of the target biomacromolecule traditionally derived from X-ray crystallographic data collected at cryogenic temperatures. Con- ventional protein X-ray crystallography is limited by possible cryo-artifacts and its inability to locate the functional hydrogen atoms crucial for understanding chemistry occurring in enzyme active sites. Neutrons are ideal probes to observe the protonation states of ionizable amino acids at near-physiological temperature, directly determining their electric charges – crucial informa- tion for drug design. Our room-temperature X-ray crystal structures of Mpro provided insights into the reactivity of the catalytic cysteine, malleability of the active site, and binding modes with clinical protease inhibitors. The neutron crystal structures of ligand-free and inhibitor-bound Mpro were determined allowing the direct observation of protonation states of all residues in a coron- avirus protein for the first time. This information was used to design nanomolar hybrid reversible covalent inhibitors with robust antiviral properties. High-throughput virtual screening, utilizing ORNL's supercomputing capabilities, in conjunction with in vitro assays identified a lead nonco- valent compound with sub-micromolar affinity. The neutron structure of Mpro in complex with the noncovalent inhibitor was used in a structure-activity relationship (SAR) study guided by vir- tual reality structure analysis to novel Mpro inhibitors with improved affinity to the enzyme. A series of X-ray structures, and biophysical and biochemical measurements on specifically designed Mpro constructs that mimic the immature monomeric state of the enzyme provide insights into the mechanism of Mpro autoprocessing, a crucial step in the SARS-CoV-2 replication cycle and a novel target for drug design. Our research is providing real-time data for atomistic design and discovery of Mpro inhibitors to combat the COVID-19 pandemic and prepare for future threats from pathogenic coronaviruses.

SARS-CoV-2 Methyltransferase ligand screening and peptide inhibitors

Presenter: Dr SPRENGER, Janina (Deutsches Elektronen Synchroton); Dr KREMLING, Viviane (DESY)

Co-authors: Dr OBERTHUER, Dominik (Deutsches Elektronen Synchroton); Dr GALCHENKOVA, Marina (Deutsches Elektronen Synchroton); Dr MIDDENDORF, Philipp (Deutsches Elektronen Syn- chroton); Dr GELISIO, Luca (Deutsches Elektronen Synchroton); Dr CARNIS, Jerome (Deutsches Elek- tronen Synchroton); Dr EHRT, Christiane (Universitaet Hamburg); Mrs KIENE, Antonia (Deutsches Elektronen Synchroton); Prof. CHAPMAN, Henry; Mr KLOPPROGGE, Bjarne (Deutsches Elektronen Synchroton); Dr YEFANOV, Oleksandr (Deutsches Elektronen Synchroton)

Content

Non-structural protein 10 (nsp10) and nsp16 are part of the SARS-CoV-2 viral RNA replication complex. Nsp16 exhibits methyltransferase activity needed for mRNA capping and is active in heterodimeric complexes with the enzymatic inert nsp10. The inactivation of this complex inter- feres severely with viral replication, making it a promising drug target against COVID-19. As only limited information on ligands binding to nsp10-nsp16 is available, we screened small compound libraries (~ 200 compounds) containing potential methyltransferase-binders that were soaked into protein crystals and the structures solved by X-ray crystallography. This has only recently become possible due to a dramatically shortened measuring time for single crystals at synchrotrons. We obtained 36 data sets of the nsp10-16 complex with purine derivatives bound to the substrate bind- ing sites. Promising compounds are being tested in binding, activity, and viral inhibition assays. In parallel, we are testing small nsp10-derived peptides that potentially disrupt the complex for- mation of nsp10-16. We see significant reduction of enzyme activity with several peptides and indications of complex disruption in small-angle-X-ray scattering (SAXS) data. Virus inhibition is currently being tested. Our results can be used for structure-based drug design to fight COVID-19 and may contain potent inhibitors of SARS-CoV-2 methyltransferases.

Coherent Hard X-ray Bio-imaging at Diamond & Diamond II.

Presenter: Dr NAVE, Colin (Diamond Light Source)

Content

Colin Nave, Lucia Alianelli, Darren Batey, Paul Quinn, Christoph Rau, Kawal Sawhney, Martin Walsh.
Diamond Light Source, Didcot, UK

The key advantage of using hard X-rays (>4keV) for imaging biological material is that it enables the study of thick specimens (>10 microns) at resolutions approaching 20 nm. This is especially important when examining features which extend over long distances such as nerve fibres in the brain. The use of coherent x-rays for such studies is a key part of the case for upgrading existing machines and constructing new brighter synchrotron sources. Due to the complexity of biological cells and tissue, it is expected that 3D images will be essential for the reliable interpretation of structures in thick specimens.

A description of the status of coherent hard x-ray bio-imaging at Diamond will be given together with plans for exploiting the increased coherent flux which will be available within the Diamond II project. This will provide a new machine lattice, up to 5 flagship beamlines and upgrades and enhancements across all instruments.

Issues to be covered will include:

- Recent developments at the coherent imaging beamline I13-1
- A dedicated coherent hard x-ray bio-imaging beamline on Diamond II
- The need for cryo-cooling for both frozen hydrated and fixed, stained material
- The use of the term resolution for studying material which includes features with a wide range of different contrast.
- Examining thick specimens beyond the depth of focus limit.
- Whether the very high time averaged coherent flux on XFEL sources could be used for coherent imaging.

The aim is to stimulate discussion about these issues.

Correlative imaging to resolve molecular structures in individual cells

Presenter: KLEMENTIEVA, Oxana (Lund University)

Content

Label-free chemical imaging of complex living systems is the holy grail of biochemical research. The current analysis techniques require extensive sample preparation, often due to the presence of interfering molecules such as water, making them unsuitable for the analysis of such systems. Here, we examined living tissues and small vertebrates at sub-micron resolution by optical pho- tothermal infrared microspectroscopy. In my talk, I will address recent advances of infrared spec- troscopy and explain how it can be used to analyze at submicron resolution fresh, unprocessed, fully hydrated tissue biopsies from diverse organs, to yield structural and compositional insights into spatiotemporal changes with relevance to diseases. I will also show that now infrared informa- tion can be extracted from living organisms, such as salamander embryos, without compromising development. Thus, demonstrating time-resolved and in situ investigation of chemical and struc- tural changes of diverse biomolecules in their native conditions.

MX3: A new macromolecular crystallography beamline at the Australian Synchrotron

Presenter: Dr ERIKSSON, Daniel (Australian Synchrotron - ANSTO)

Primary authors: Dr ERIKSSON, Daniel (Australian Synchrotron - ANSTO); Dr CARADOC-- DAVIES, Tom (Australian Synchrotron - ANSTO); Mr BARROW, Graham (Australian Synchrotron - ANSTO); Dr CAIN, Nicole (Australian Synchrotron - ANSTO); Dr CAMPBELL, Eleanor (Australian Syn- chrotron - ANSTO); Dr CHEN, Simin (Australian Synchrotron - ANSTO); Mr CHERUKUVADA, Hima (Australian Synchrotron); Dr HERNANDEZ VIVANCO, Francisco (Australian Synchrotron - ANSTO); Mr KHEMARAM, Rakesh (Australian Synchrotron - ANSTO); Mr OLDFIELD, Jacob (Australian Synchrotron
- ANSTO); Mr QUINTON, Joe (Australian Synchrotron - ANSTO)

Content

The High-Performance Macromolecular Crystallography beamline (MX3) will be capable of pro- viding high-flux, microfocus X-rays for small and weakly diffracting protein crystals. The beamline has an energy range from 10-15 keV with beam sizes at sample from 2x2 to 40x40 microns with flux up to 1.3e14 ph/s (at 13 keV). The beamline will provide three modes of data collection: goniometer (standard pin-mounted experiments), serial crystallography (fixed target and injector), and in-tray collection. The optical design allows users to rapidly change beam size by a combination of a secondary source aperture and compound refractive lenses.

The major endstation components are comprised of an Eiger2 XE 16M detector (Dectris), an ISARA sample changing robot (Irelec), and a MD3-up diffractometer (Arinax). This allows for data collec- tion in excess of 500 Hz via the Dectris Stream2 interface, rapid automated pin and tray exchange, and automated pin centering with a sub-micron sphere of confusion.

A high degree of automation will support unattended data collection of entire projects. Given a set of expected outcomes, all samples attached to an experiment can go through screening, data collec- tion and merging without user interaction. Results are entered in a database for easy comparisons across multiple experiments.

In-tray screening and in-tray data collection are related but separate. In-tray screening will be achieved through a program aimed at synchrotron users and non-users alike. “Tray Tuesdays” will set aside beamtime for automated screening and evaluation of plate wells. The ScreenShot user interface developed in-house will serve as a portal for sending trays and evaluating the results. Early X-ray diffraction screening of crystalline material can direct researchers’ efforts towards the most promising conditions, guiding optimisation and minimising wasted synchrotron time.

In-tray data collection in contrast, aims to collect entire datasets without harvesting of crystals. This will be performed at room temperature and will merge data from multiple crystals to form a complete dataset. While screening can be done with most standard tray types, colleting full datasets requires special low-background trays that are commercially available.

The FragMAX facility for structure-based drug discovery at MAX IV Laboratory

Presenter: KROJER, Tobias (BioMAX)

Co-authors: JAGUDIN, Elmir; NAN, Jie (MX-group); GOURDON, Maria (Lund Protein Production Platform, LP3); EGUIRAUN, Mikel; GORGISYAN, Ishkhan; GONZALEZ, Ana (MAX IV); KNECHT, Wolfgang (Lund University); Dr OBIOLS, Marc (Industry Office - MAX IV); LARSSON, Magnus (MAX IV Laboratory); THUNNISSEN, Marjolein (Lund University); Dr UWE, Müller (HZB); URSBY, Thomas (MAX IV Laboratory)

Content

The FragMAX facility supports structure-based drug and chemical tool compound discovery at MAX IV Laboratory. It was designed as a platform for crystal-based fragment screening, but the underlying workflows are applicable to all medium- to large-scale protein-ligand studies. The platform is comprised of three primary elements: (i) a medium-throughput crystal preparation facility, (ii) diffraction data collection at the BioMAX beamline, and (iii) FragMAXapp, an intuitive web-based tool for large-scale data processing. In 2019, the FragMAX platform began providing services to external users and has since established an international user program that is accessible to academic and industrial research organizations. Access can be requested through the MAX IV user program, the MAX IV Industrial Relations Office and iNEXT Discovery.

The FragMAX crystal preparation facility is co-located with the Lund Protein Production Platform (LP3) and provides the complete set of instruments for protein crystallization and crystal optimiza- tion. In addition, it offers workflows that include liquid handling systems for automated crystal soaking and robot-assisted crystal mounting. The facility provides free access to multiple fragment libraries, including the in-house developed FragMAXlib, and allows users to send their own com- pound collections or screening sets. FragMAX personnel can help with sample preparation, and users are also welcome on-site. Data collection is performed at the BioMAX beamline which offers a high-intensity X-ray beam, fast and reliable sample changer (464 crystals), state-of-the-art X-ray detector (Eiger 16M) and, as of spring 2023, fully unattended operation. All steps of the experiment, from crystal preparation to structure refinement, are recorded in a transferable database system. In addition, FragMAX provides several freely accessible tools for accelerated structure modeling and refinement as well as PDB deposition support. FragMAX provides customized experiments and a modular experimental design, allowing users of varying levels of expertise to routinely ob- tain actionable screening hits for their targets. All workflows are continuously evolving, and we intend to increase cooperation with other Swedish research infrastructures in the future.

Routine room temperature protein structure determination in situ at Diamond beamline VMXi: current status and recent developments

Presenter: Juan Sanchez-Weatherby

Authors: Mike Hough[1]; Amy Thompson[2]; Hans Pfalzgraf[2]; Richard Gildea[2]; Halina Mikolajek[2]; James Sandy[2]; Juan Sanchez-Weatherby[2]

[1] *Diamond Light Source*

[2] *Diamond light source*

Corresponding Authors: juan.sanchez-weatherby@diamond.ac.uk, hans.pfalzgraf@diamond.ac.uk, halina.mikolajek@diamond.ac.u james.sandy@diamond.ac.uk, amy.thompson@diamond.ac.uk, michael.hough@diamond.ac.uk, richard.gildea@diamond.ac.uk

The need for rapid turnaround times from the appearance of protein crystals to the determination of high quality crystal structures has driven many aspects of automation. Moreover, it is increasingly recognised that room temperature structures allow access to protein dynamics, may reveal ligand binding modes not apparent at cryogenic temperatures and allow for time resolved structures to be determined. We present the current pipeline of the Crystallisation Facility at Harwell and the Micro Focus, pink beam Diamond Beamline VMXi 1 and the capability of the pipeline to produce high quality structures at room temperature from small numbers of crystals in situ, for example within crystallisation plates. Protein samples in solution are provided by users, with automated crystalli- sation and data collection offering datasets and structure solution in many cases within days. In situ data collection allows for rapid feedback on crystallisation conditions for further optimisation and is particularly suitable for crystals challenging to cryo-protect or that are not robust to mechan- ical handling or other stresses. Operation is typically completely remote with data measured using a queueing system, providing convenience for the scientist. Recent developments in sample deliv- ery, crystal identification and multi-crystal data analysis [2] will also be described. Finally, we will present recent developments in combining serial crystallography using a tape drive sample delivery method (with drop on drop mixing) and X-ray emission spectroscopy at VMXi.

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A high-resolution correlative light and X-ray 3D cryo-imaging platform for cells and tissue at near-native physiological conditions.

Presenter: Dr JADHAV, Archana (Diamond Light Source Ltd)

Co-authors: Dr OKOLO, Chidinma (Diamond Light Source Ltd); Dr NAHAS, Kamal (Diamond Light Source Ltd); Mr FISH, Thomas (Diamond Light Source Ltd); Mr TAYLOR, Adam (Diamond Light Source Ltd); Prof. HARKIOLAKI, Maria (Diamond Light Source Ltd)

Content

High-resolution imaging of the cellular world in recent years has underpinned a revolution in high-content volume data accumulation resulting in a new understanding of cell organization and behaviour. Amongst the highlights of microscopy developments in cellular imaging has been the emergence of new correlative imaging modalities that incorporate Soft X-ray Tomography (SXT) of cryopreserved cells and tissue. At the correlative cryo-imaging beamline B24 of the UK synchrotron, we have devised and refined such a correlative imaging platform to a high level of automation and throughput by focusing not only on technique development but also on the acces- sibility of ease or use.

SXT is a high-resolution 3D mesoscale imaging technique for cells and tissues up to 12 µm in thick- ness in the fully hydrated state without using chemical fixation, sectioning or milling, or the use of contrast-enhancing agents. Under cryogenic conditions, vitrified samples are irradiated with 'water-window' X-rays to achieve a lateral resolution of structures within cells to 25 nm depend- ing upon the optical set-up of the SXT microscope. At B24, a 3D super-resolution fluorescence microscope based on structured illumination microscopy (SIM) principles was developed as a com- plementary tool to match molecular localisation with high-resolution structural data acquired in SXT under cryogenic conditions leading to the development of a unique same-sample correlative light and X-ray tomography (CLXT) platform.

Here we will highlight representative B24 data from current biomedical areas and disease devel- opments in both methodology and applications alongside new results in infection and immunity that have been only recently discovered through the CLXT platform.

Applications of density reconstruction from solution scattering to drug discovery

Presenter: GRANT, Thomas (University at Buffalo)

Co-authors: Dr CHAMBERLAIN, Sarah (University at Buffalo); Mr SINGH, Jitendra (University at Buffalo)

Content

Protein-ligand interactions play a pivotal role in drug discovery and understanding biological pro- cesses. Structural biology techniques, such as X-ray crystallography, offer atomic-level insights into these interactions in a static environment. However, in solution, ligand binding can cause proteins to adopt multiple conformations that differ from those observed in crystals, making it challenging to obtain accurate structures. X-ray solution scattering (XSS) is a valuable tool for studying proteins in solution due to its ability to provide complementary information to high-resolution techniques, and it can be performed in high throughput at modern X-ray sources. In this presentation, we describe our algorithm called DENSS, which reconstructs *ab initio* density maps directly from XSS data. We discuss our latest developments in DENSS, which enable us to probe the structure and dynamics of protein-ligand complexes in solution with substantially improved resolution. Our developments, coupled with the ability to perform XSS at room temper- ature and in high throughput, represent a significant advancement in the field of structural biology and drug discovery. Overall, our algorithm offers a valuable tool for studying protein-ligand in- teractions in solution, where biological processes take place. Our approach enables researchers to probe the structure and dynamics of proteins and their ligands at higher resolution, facilitating the design of more effective drugs.

TOLL receptor Serial Femtosecond Crystallography structure

Presenter: Dr DARMANIN, Connie (La Trobe University)

Primary authors: Dr DARMANIN, Connie (La Trobe University); Dr HOLMES, Susannah (De- partment of Mathematical and Physical Sciences, School of Engineering, Computing and Mathematical Sciences, La Trobe University,); Prof. KOBE, Bostjan (School of Chemistry and Molecular Biosciences, The University of Queensland,); Dr VE, Thomas (Institute for Glycomics, Griffith University); Dr MAR- TIN, Andrew (School of Science, RMIT University,); Dr AQUILA, Andrew (Linac Coherent Light Source, SLAC National Accelerator Laboratory); Prof. ABBEY, Brian (Department of Mathematical and Physical Sciences, School of Engineering, Computing and Mathematical Sciences, La Trobe University)

Content

Understanding the dynamics of biological processes provides new insight into treating disease. X-ray crystallography reveals what proteins look like at the atomic scale and has been key for developing new therapeutics. TOLL-like receptors are activated by pathogens, which results in a string of protein interactions within the cell producing an immune response. Two responses can occur, immunity, or inflammation. Inflammation is associated with several pathological states, in- cluding infectious, autoimmune, inflammatory, cardiovascular, and cancer-related disorders which makes them an important biological target. TOLL like receptor adaptor proteins when mixed can oligomers into highly ordered structures. Using X-ray Free Electron Laser (XFEL) facilities and their extremely intense sources of X-rays Serial Femtosecond Crystallography (SFX) data was col- lected on these oligomers. Using SFX methods we have solved the first biological relevant structure of Myeloid differentiation primary response gene 88 (MYD88), a Toll-like receptor (TLR) adaptor protein, which plays an important role in inflammatory disease. The data generated at the Linac Coherent Light Source provided structural and mechanistic insight into TLR signal transduction.

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Importance of Validation in SAXS/SANS

Presenter: Prof. TREWHELLA, Jill (The University of Sydney)

Content

The biomolecular SAS community of users has grown sharply since the 1990's with increased ease of access and innovation in instrumentation and analysis tools. With this growth, SAS methods have become attractive to mainstream structural biologists who traditionally have relied almost exclusively on high-resolution, data-rich techniques. Key to this development have been the mod- elling tools for calculating SAS profiles from atomic coordinates that bridge the gap between high resolution structure and solution scattering. At the same time, there have been important com- munity efforts (reviewed in 1) to develop and agree standards for data quality, model validation, publication, and data sharing with the aim of ensuring transparency and completeness in report- ing so that the broader structural biology community can be confident in assessing and using the results of biomolecular SAS experiments. The biomolecular SAS community now has a set of com- prehensive guidelines for publication of biomolecular SAS [2] with standard reporting templates [3]. In a major new development, we also now have a core consensus set of SAXS and SANS profiles on five proteins against which existing and new methods for SAS profile calculation from atomic coordinates can be benchmarked [4]. This consensus data set was derived from 171 SAXS and 76 SANS profiles collected on 12 small-angle X-ray scattering (SAXS) and four small-angle neutron scattering (SANS) instruments across the Americas, Europe and Asia and provided an important quantitative assessment of SAS data reproducibility. The value of adherence to the 2017 guide- lines for publication of biomolecular SAS and 3D modelling was well demonstrated in this study. Further, while calculation of SAS profiles from atomic coordinates for a current set of popular methods show good general agreement with the consensus profiles, there are residual differences that offer the opportunity for evaluating, comparing and potentially improving any one approach to theoretical SAS profile prediction.

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Abstracts Poster Presentations

1

Solvation Shell Structure of Proteins probed by Small- and Wide- Angle X-Ray Scattering

Authors: Orion Shih[1]; Yi-Qi Yeh[1]; Kuei-Fen Liao[1]; Je-Wei Chang[1]; Chen-An Wang[1]; Bradley Mansel[1]; Chun-Jen Su[1]; Wei-Ru Wu[1]; U-Ser Jeng[1]

[1] *NSRRC*

Corresponding Author: shih.orion@nsrrc.org.tw

Small- and wide-angle X-ray scattering (SWAXS) data provides information about the shape and size of bio-macromolecules in solution. The newly developed BioSAXS endstation at the beamline 13A of the Taiwan Photon Source features an in-vacuum SWAXS detecting system comprising two area detectors (Eiger X 9M/1M) and an online size-exclusion chromatography system incorporated with several optical probes, including UV-vis absorption spectrometer and refractometer. The in- strumentation allows simultaneous SAXS and WAXS data collection with a high signal-to-noise ratio, enhancing structural studies of biomolecules by accessing finer details of solution structures. Here we tested a model protein BSA with two common denaturants: urea and guanidinium chloride (GdmCl). The intradomain interaction loss is clear around the middle q region. The high q peak (~1.5 Å-1) was previously assigned to the secondary structure but is still present when the protein is fully denatured. We showed how the solvation structure changes correlate with the high q peak change and discussed the different outcomes from urea and GdmCl, suggesting a dissimilar denaturation mechanism.

2

Differentiating the solution structures and dimer-dimer interfacial dynamics of transthyretin stabilized by tolcapone and tafamidis

Authors: Orion Shih[1]; Yu-Chen Feng[None]; Sashank Agrawal[None]; Kuei-Fen Liao[None]; Yi-Qi Yeh[None]; Je-Wei Chang[None]; Tsyr-Yan Yu[None]; U-Ser Jeng[None]

[1] *NSRRC*

Corresponding Author: shih.orion@nsrrc.org.tw

Human transthyretin (TTR) is a homotetrameric protein that transports thyroxine (T4) and retinol- binding protein (RBP) in the serum and cerebrospinal fluid. Dissociation of the tetramer form of TTR is believed to result in monomer aggregation into the several identified fatal TTR amyloidosis forms. In the past, several synthesized small molecules were developed to bind and stabilize the dimer- dimer interface of the TTR tetramer as a therapeutic strategy, thereby preventing the dissociation of the tetramer and consequent amyloid fibrilliazation. In this study, using small- and wide-angle X-ray scattering (SWAXS) we analyze the solution structures of TTR and TTR bound respectively with the two therapeutic molecules of tolcapone and tafamidis, in solutions containing different urea concentrations. Our results show that when the binding ratio between the small molecule to TTR is two to one, both tolcapone and tafamidis can preserve the tetramer structure of TTR well up to 8 M urea at 37 oC; however, tolcapone exhibits a better-stabilizing effect than tafamidis, when the binding ratio reduces to 1:1. We also probe the changes of the dynamics of the dimer-dimer interface of TTR upon the binding of the small molecules using NMR, to reveal possible differentiations in the binding dynamics of the two small molecules. These results provide hints on developing a cocktail strategy by adding natural compounds, such as curcumin, to reduce the dosage of the chemical compound in the therapy of TTR amyloidosis.

3

Detecting SARS-CoV-2 in saliva by Small-angle X-ray Scattering

Author: Andre Conceicao[1]

Co-authors: Ronald von Possel [2]; Petra Emmerich [2]; Sylvio Haas [3]

[1] *Deutsches Elektronen-Synchrotron DESY*

[2] *Bernhard-Nocht-Institut für Tropenmedizin*

[3] *DESY*

Corresponding Authors: sylvio.haas@desy.de, emmerich@bnitm.de, vonpossel@bnitm.de, andre.conceicao@desy.de

SARS-CoV-2, the causative agent of the COVID-19 pandemic, has speeded its spread across the globe immediately after its emergence in China in December 2019. Since then, a coordinated and tireless race for developing simple, less invasive, cheap, highly accurate, and able to be applied in large-scale tests to supply the huge demand of governments to detect infected persons and plan effective isola- tion strategies was initiated. Apart from this pandemic, which caused ~6.8 M deaths worldwide, the human population has suffered from many pandemics throughout history. Although in several aspects the worldwide policies to reduce the risk of epidemics have had some success, the International Health Relations (IHR) committee concluded that the world is still not well prepared to respond to a severe pandemic. The IHR has required every country for comprehensive preparedness in develop- ing core capacities to prevent, detect, and respond to public health events. Thereby, here we present an approach based on using small-angle x-ray scattering to analyze saliva specimens combined with an automated pipeline exploiting machine learning to enable massive screening of the population for COVID-19.

The SARS-CoV-2 virus was isolated and by gamma-radiation inactivated at the Bernhard Nocht In- stitute for Tropical Medicine (BNITM), Hamburg. A serial dilution of the virus into two synthetic salivas (from Sigma Aldrich) was performed: 1:10, 1:100, 1:3000, and 1:20000. Except for the highest concentrated solution, the other concentration solution was chosen to represent the minimum, av- erage, and maximum virus concentration observed in SARS-CoV-2 positive diagnosed patients in a large hospital in Germany.

Synchrotron SAXS data were collected at the P62 beamline (E= 12 keV) at the PETRA III storage ring, in Hamburg, Germany. The X-ray beam (600 x 300 µm2) is focused on the capillary filled up with the samples. The sample-to-detector distance was chosen to obtain a q-range of 0.01 nm-1 to 0.98 nm-1. Five SAXS images were collected for each sample every 1s. These images were summed up to obtain a better signal-to-noise ratio.

A diagnostic model based on principal component analysis of the linear fitting parameter at the q-range between 0.08 and 0.2 nm-1 was developed. Naive Bayes (NB) was used to classify the sam- ples as positive or negative for SARS-CoV-2. The approach was tested by performing it directly on radiation-inactivated viruses in saliva specimens and values of sensitivity, specificity, and accuracy of 87.50%, 100.00%, and 91.67%, respectively, were obtained. Due to no additional sample prepara- tion steps and short acquisition times, significant savings in time and cost can be achieved with the proposed method.

4

Macromolecular crystallography in situ data collection strategies at a second-generation synchrotron

Author: Leonard Chavas[1] [1] *Nagoya University*
Corresponding Author: l.chavas@nusr.nagoya-u.ac.jp

In situ macromolecular crystallography is a technique used in structural biology to determine the three-dimensional structure of biomolecules without the need for subsequent handling or modifi- cation of the crystals that are operated in their native environment. Exposing macromolecular ob- jects to strong X-ray radiation causes significant damage that has a noticeable impact on the quality and accuracy of the data that can be collected on the crystals under study. Among the diverse approaches applied to minimize the effects of radiation damage, exposing the crystals to low-intensity X-ray beams and short exposure times assists in elongating the life of the exposed samples. The combination of both strategies is especially efficient when using high-speed reading detectors, more widely used at macromolecular crystallography beamlines. The second and third-generation syn- chrotrons mainly differ in the method that is used for producing and providing the X-ray photons used for the experiments. Although second-generation synchrotrons are still used and offer high- quality X-ray beams, they remain restricted in terms of brightness, coherence, and beam tunability. In the current presentation, we aim to highlight how macromolecular crystallography beamlines at second-generation synchrotrons could be optimized for the industrial development of in situ protein crystallography experiments.

5

Characterization of Aquaporin Containing Vesicles via Electron Microscopy, Light- and Small Angle Scattering

Authors: Zsófia Szathmáry[1]; Jacob Kirkensgaard[1] [1]
Copenhagen University
Corresponding Authors: jjkk@food.ku.dk, zes@food.ku.dk

Water is essential for all life forms on the planet, yet after thousands of years in which it has been an abundant resource, the situation is drastically changing to the point where water scarcity has become one of the greatest threats of the 21st century (1). Membrane filtration technologies are able to provide reliable filtered water qualities and a low footprint meanwhile presenting a high degree of automation which is essential for scalability (2). Inspired by nature, a new generation of mem- branes have been gaining a particular interest. Biomimetic membranes combine the mechanisms of cellular water transport via transmembrane proteins (aquaporins) with classical synthetic mem- brane technologies (Figure 1) (3). Even though the technology is extremely promising, preparation of biomimetic membranes is not a trivial task, as aquaporins need to be stabilized in an artificial environment that mimics the natural cell membrane (4).

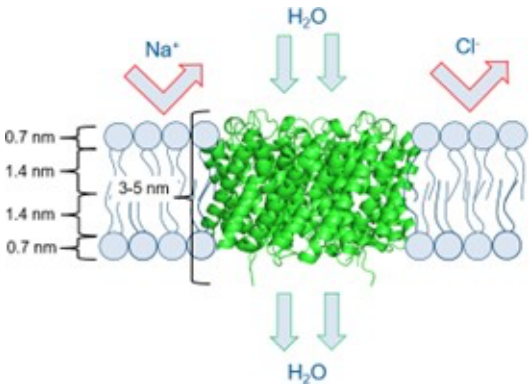

Figure 1: Figure 1: Schematics of the lipid bilayer embedded AqpZ protein transport

Figure 1: Schematics of the lipid bilayer embedded AqpZ protein transport

In the presented work, a variety of analytical techniques are used to characterize the incorpora- tion of aquaporin proteins in different vesicle systems, mimicking the cellular bilayer. The two types of vesicle materials in focus are natural lipids and synthetic polymers. Liposomes provide a native-like membrane environment with their lipid bilayer structure, but lack physical and chemical stability, which is a problem for most industrial applications (5). Polymersomes on the other hand, are made of block copolymers and offer greater physical and chemical stability with the opportunity for customizations. However, the reconstitution of aquaporin proteins into polymersomes, while maintaining their structure and functionality, poses great challenges (6). Different tools such as Electron Microscopy, Light-and Small Angle Scattering are used to obtain structural information on the nanocarriers as well as the protein incorporation within the different types of bilayers. While Electron Microscopy can provide mostly qualitative data, Stopped-Flow Light Scattering has the potential of producing dynamic results of real-time water transport. In combination with changes of the scattering profiles that Small Angle Scattering methods can detect, information regarding the vesicles' size and shape can be derived, while via contrast variation the bilayer material can be distinguished from the protein, allowing for a quantitative assessment of aquaporins within the vesicles.

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6

Extending the DIALS toolkit

Author: David Waterman[1]

Co-author: David McDonagh [1 1] *UKRI*
STFC
Corresponding Authors: david.mcdonagh@stfc.ac.uk, david.waterman@stfc.ac.uk

DIALS is a data processing package for single crystal diffraction. It was written initially to serve the needs of large scale X-ray synchrotron and XFEL facilities and developed as an open source, collaborative project to provide maximum benefit to the community. Over the past decade, DIALS has developed into a mature package and provides the backbone for autoprocessing pipelines run at Diamond's MX beamlines and other places. The initial scope, covering analysis from images to integrated intensities, has been extended to include data scaling and multi-crystal analysis. The extensible *toolkit* design of the software has also allowed us to explore new use cases. Some of these will be presented, including plugins for reading new image formats, tools developed to cope with electron diffraction data, and now elements of Laue data processing, both for neutron time-of-flight and X-ray Laue diffraction.

7

Investigating the Monomer-Dimer alterations of SARS-CoV-2 3- CL Protease

Author: MOHIT BHARDWA **Co-author:** Ashok Kumar Patel [1]

[1] *Kusuma School of Biological Sciences, Indian Institute of Technology Delhi*

Corresponding Author: mohit.bhardwaj@bioschool.iitd.ac.in

Recently the world has been ravaged by the outbreak of severe acute respiratory syndrome coron- avirus 2 (SARS-CoV-2). SARS-CoV-2 belongs to the Coronaviridae family, Coronaviruses (CoVs) are positive-sense single-stranded RNA viruses with a 5′ Cap and a 3′ poly-A tail that may infect both human beings and animals. SARS-CoV-2 maturation, replication, and invasion all rely on the viral polyproteins (pp1a and pp1ab) being cleaved by a cysteine-rich 3C-like protease(3CLpro), as that is its primary function. It has been reported that 3CLpro is catalytically active as a dimer and each pro- tomer contains a catalytic dyad (His41/cys145). It is well known that the N- and C-terminal domains were crucial in regulating the monomer/dimer equilibrium of 3CLpro and its catalysis. Unconven- tional approaches have been explored to design therapeutic agents against 3CLpro as it has highly conserved sequences: Direct inhibition of the catalytic site by employing compounds targeting the substrate binding pocket; and Reduction of the catalytic activity by targeting the dimerization in- terface. The elucidation of dimerization pathways is crucial for the structure-based development of novel therapeutics for infectious diseases caused by coronaviruses. Our research intends to identify the crucial function of long-distance interactions within the protease as well as to clarify whether dimerization is unquestionably required to activate 3CLpro catalysis. Our study demonstrates the mechanism by which the enzyme alters its structure in response to the loss of dimerization and the structural modifications that result in the inactivation of the catalytic activity. Based on the structural investigation, we mutated amino acids at crucial regions of the dimer interface to test their relevance in oligomerization and activity. We checked the oligomerization by Size Exclusion Chromatography and Native-PAGE. The result for the oligomerization was also confirmed by the enzyme activity. Also, compare the effect of mutants on the thermal stability by Differential scan- ning calorimetry and Circular Dichroism thermal. From our initial results, a certain single amino acid is a crucial part of sustaining dimerization; as a result, its mutation abruptly converted the enzyme into an inactive monomeric form. The structural study of the mutants will demonstrate a specific amino acid's crucial function in retaining the active site's internal structure and in aligning important residues associated with binding at the dimer interface and substrate catalysis.

8

Visualizing protonation states in serine hydroxymethyltransferase with neutron crystallography

Authors: Victoria, N. Drago[1]; Claudia Campos[2]; Aliyah Collins[2]; Mattea Hooper[2]; Oksana Gerlits[2]; Kevin, L. Weiss[1]; Matthew, P. Blakeley[3]; Robert, S. Phillips[4]; Andrey Kovalevsky[1]

[1] *Neutron Scattering Division, Oak Ridge National Laboratory*

[2] *Department of Natural Sciences, University of Tennessee Wesleyan*

[3] *Large Scale Structures Group, Institut Laue-Langevin*

[4] *Department of Chemistry; Department of Biochemistry and Molecular Biology, University of Georgia*

Corresponding Authors: kovalevskyay@ornl.gov, plp@uga.edu, blakeleym@ill.fr, ogerlits@tnwesleyan.edu, dragovn@ornl.gov, weisskl@ornl.gov

Serine hydroxymethyltransferase (SHMT) is a pyridoxal-5'-phosphate (PLP) dependent enzyme that catalyzes the tetrahydrofolate (THF)-dependent cleavage of L-Ser to form glycine and 5,10-methylene- THF. This reaction is significant for its role in the biosynthesis of thymidine and purines, as well as the methyl group of methionine by providing single carbon units to one-carbon metabolism. Human mitochondrial SHMT (hSHMT2) is overexpressed in a multitude of cancers and is acknowledged as a significant target for anti-cancer therapeutics. Here, we present a 2.3 Å joint X-ray/neutron (XN) structure of the homodimeric SHMT from *Thermus thermophilus* (*Tth*SHMT), whose active site is conserved compared to that of hSHTM2, in the open conformation depicting the PLP cofactor co- valently bound to the catalytic lysine in the internal aldimine state and a sulfate ion occupying the substrate binding site. In addition, a second joint XN structure obtained by soaking a *Tth*SHMT crys- tal with L-Ser revealed the substrate bound at the entrance of the active site in a solvent-exposed shallow pocket in a pre-Michaelis complex while the sulfate anion continues to block the active site. We further tracked the substrate through the active site by obtaining an X-ray structure of a pseudo-Michaelis complex by soaking a *Tth*SHMT crystal with D-Ser, a non-reactive substrate enan- tiomer. Nuclear density maps revealed the positions of hydrogen atoms and provided the ability to accurately assign the protonation states for the amino acid residues, L-Ser substrate, and the PLP cofactor. By direct observation of the locations of hydrogen atoms and tracking substrate positions, our study provides unique atomic-level understanding of the SHMT active site that sheds new light on the enzyme's catalytic mechanism and can be employed to advance the design of anticancer drugs targeting hSHMT2.

9

X-ray scattering methods to image bone healing around bio-resorbable implants

Author: Irene Rodriguez Fernandez[1]

Co-authors: Thomas Bretschneider [2]; Omer Suljevic [3]; Nicole Sommer [3]; Tilman Grünewald [4]; Annelie Weinberg [3]; Helga Lichtenegger [2]; Andreas Menzel [1]

[1] *Paul Scherrer Institut, Villigen PSI, 5232, Switzerland*

[2] *Institute of Physics and Materials Science, University of Natural Resources and Life Science (BOKU), 1190 Vienna, Austria*

[3] *Department of Orthopedics and Traumatology, Medical University of Graz, Graz, Austria*

[4] *Aix-Marseille Univ, CNRS, Centrale Marseille, Institut Fresnel, 13013 Marseille, France*

Corresponding Authors: irene.rodriguez-fernandez@psi.ch, omer.suljevic@medunigraz.at, helga.lichtenegger@boku.ac.at, andreas.menzel@psi.ch, anneliemartina.weinberg@medunigraz.at, nicole.sommer@medunigraz.at, tilman.grunewald@fresnel.fr, thomas.bretschneider@boku.ac.at

In recent years, bio-resorbable magnesium implants have gained interest as treatment options for bone fractures. Their advantages include their mechanical properties, their excellent immunological response during healing, and the reduced need for a second intervention to remove the implant. In this study, we wanted to understand the behavior of bone around Mg biodegradable implants and the impact that physical training has on bone healing and mineralization.

X-ray scattering techniques are well suited for this application as they probe structural information on materials with features on the nanometer scale. Small-angle X-ray scattering (SAXS) can yield information about the changes in orientation of bone nanostructure. Small-angle scattering tensor tomography (SASTT) can provide spatially resolved 3D anisotropy information 1. By combining both techniques, we can obtain 3D information and mitigate SASTT's significant sampling require- ments, improving both statistics and spatial resolution.

With the use of these two techniques, we have been able to characterize the role training plays in healing and remodeling of bone around ultra-high purity (XHP) Mg implants. Our measurements suggest that physical training leads to a higher degree of orientation of hydroxyapatite (HA) in bone and found that training might lead to a quicker response of the bone metabolism. These results are highly relevant for understanding degradable implants' behavior and are expected to be of clinical significance in the treatment of bone fractures.

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SAR analysis of S1P5 receptor structure in complex with a selective inverse agonist

Authors: Alexey Mishin[1]; Elizaveta Lyapina[2]; Egor Marin[2]; Gusach Anastasiia[2]; Aleksandra Luginina[2]; Valentin Borshchevskiy [2]; Vadim Cherezov[3]

[1] *Moscow Institute of Physics and Technology*

[2] *Research Center for Molecular Mechanisms of Aging and Age-related Diseases, Moscow Institute of Physics and Technology, Dolgoprudny 141701, Russia.*

[3] *Bridge Institute, Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA*

Corresponding Author: mishinalexej@gmail.com

The sphingosine-1-phosphate (S1P) is a bioactive lysophospholipid that acts through five different subtypes of G protein coupled S1P receptors (S1PRs) - S1P1-5. S1P5 affects many cellular processes such as division, migration and survival. To date, several drugs (such as fingolimod 1, siponimod [2] and ozanimod [3]) have been developed for the treatment of multiple sclerosis, Crohn's disease and other autoimmune diseases that target S1P receptors, but they lack selectivity for receptor subtypes and mechanisms of action, which leads to adverse side effects.

Here we report the 2.2 Å crystal structure of the human S1P5 receptor in complex with its selective in- verse agonist ONO-543060, obtained by room temperature serial femtosecond crystallography (SFX) data collection at the Pohang Accelerator Laboratory X-Ray Free Electron Laser (PAL-XFEL) using sub-10 μm crystals [4].

The structure displays a distinctive binding mode for the ligand, featuring an allosteric binding sub- pocket that does not only define the subtype specificity but also presents a template for rational drug design. Together with the previously published S1P receptor structures, the newly obtained inverse agonist-bound structure provides insights into the activation mechanism and uncovers molecular mechanisms responsible for inverse agonism in the S1P receptor family.

To lay out the groundwork for future personalized medicine approaches, we mapped the known missense Single Nucleotide Variations (SNVs) from gnomAD and COSMIC genome databases on the obtained S1P5 structure and annotated their potential functional roles.

Additionally, we compared the predictive performance of S1PR AlphaFold2 models for virtual ligand screening with experimental structures and found that, despite close structural similarity, the crys- tal structures better capture the full details of specific signaling states, while the S1PR AlphaFold2 models display mixing features of different functional states.

The high-resolution S1P5 structure provides a template for the structure based design of lead or tool compounds and our analysis provides insights into possible therapeutic strategies for the treatment of disorders related to the S1P family. The work was supported by the Russian Science Foundation (project no. 22-74-10036; https://rscf.ru/project/22-74-10036/).

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CCP4 8.1 - the next major release of the CCP4 suite

Authors: Charles Ballard[1]; Ville Uski[1]

Co-authors: Maria Fando [1]; Ronan Keegan [1]; Andrey Lebedev [1]; Eugene Krissinel [1]; David Waterman [1] *UKRI-STFC (CCP4)*

Corresponding Authors: andrey.lebedev@stfc.ac.uk, maria.fando@stfc.ac.uk, david.waterman@stfc.ac.uk, charles.ballard@stfc.ac.u ronan.keegan@stfc.ac.uk, ville.uski@stfc.ac.uk, eugene.krissinel@stfc.ac.uk

Autumn 2023 will see the next major release of the CCP4 software suite. Following the, largely, suc- cessful update from python2 to python3 for the 8.0 series, 8.1 will use a python 3.9 base. Among the step changes is the move to DIALS 3.13+ {1] with an updated graphical user interface, DUI2, greater coot scripting from Paul Emsley [2], the introduction of webCoot (code name moorhen) [3], plus developments from the Phaser team in Cambridge, and finally native M series processor support on Macs. In the meantime the planned monthly update schedule [4] to 8.0 will see further developments on the use of predicted models, CCP4cloud [5] and CCP4i2 [6].

The suit provides automated pipelines and tools for the macromolecular structure solution process, including for example XIA2 for data-processing, MRBump and ARCIMBOLDO for molecular re- placement, CRANK2 for experimental phasing, Modelcraft for model building. There is continued development of tools to use predicted models in phasing (MRParse, SliceNDice), model building and validation (Conkit). The CCP4cloud interface can be integrated into a facilities services to offer more fine grained control, and provide a “one stop shop” for the structure solution.

1 http://dials.github.io

[2] https://github.com/pemsley

[3] http://moorhen.org

[4] http://www.ccp4.ac.uk/?page_id=3088

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[6] https://ccp4i2.gitlab.io/rstdocs/updatelog/index.html

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New developments in CCP4 to tackle the phase problem in Macro- molecular Crystallography

Author: Ronan Keegan[1]

Co-authors: Charles Ballard [2]; Stuart McNicholas [3]; Adam Simpkin [4]; Luc Elliott [4]; Daniel Rigden [4]

[1] *UKRI-STFC*

[2] *CCP4*

[3] *York University*

[4] *University of Liverpool*

Corresponding Authors: drigden@liverpool.ac.uk, ronan.keegan@stfc.ac.uk, l.elliott3@liverpool.ac.uk, charles.ballard@stfc.ac.uk, hlasimpk@student.liverpool.ac.uk, stuart.mcnicholas@york.ac.uk

The success of Deepmind in CASP14 has resulted in a new era in structural biology where highly accurate predictions for macromolecular structures can be generated computationally in just a few minutes or less. This has presented new challenges and opportunities to the experimental struc- tural biology community. With the availability of accurate predictions, solving the phase problem in macromolecular crystallography through the molecular replacement technique (MR) has become much more tractable. Despite this, performing MR using a predicted model is not straight forward in many cases. Differences in the relative conformations of domains in a prediction and what has been crystallised, as well as low confidence in predicted residues, can hinder MR and result in difficulties determining a solution. Other problems can also stifle resolving the phases, such as the problem of crystal contamination. Here we present several new applications that have been developed and made available through the CCP4 suite to help with optimising the chances of successful MR when using a predicted model as well as tackling the problem of contamination. MrParse 1 is designed to assist users in searching for and preparing MR search models from both the PDB and the new and vast EBI Alphafold and ESMAtlas prediction databases. Slice'N'Dice [2], an automated MR pipeline, makes use of machine learning clustering algorithms to identify domains or tightly grouped clusters of atoms in a predicted model that are likely to make suitable MR search models. Developed to tackle the problem of contamination, SIMBAD [3] is a sequence-independent automated MR pipeline that can be used to quickly test a processed X-ray dataset for possible contamination during crystallisation.

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Automation of high-throughput MX beamline at SPring-8

Authors: Masaki Yamamoto[1]; Kunio Hirata[1]; Hiroaki Matsuura[1]; Naoki Sakai[2]; Seiki Baba[2]; Nobuhiro Mizuno[2]; Yuki Nakamura[3]; Hironori Murakami[2]; Kazuya Hasegawa[2]; Takashi Kumasaka[2]

[1] *RIKEN SPring-8 Center*

[2] *Japan Synchrotron Radiation Research Institute (JASRI)*

[3] *Japan Synchrotron Radiation Research Institute (JASRI)*

Corresponding Authors: hiroaki.matsuura@riken.jp, kumasaka@spring8.or.jp, baba@spring8.or.jp, yamamoto@riken.jp, nsakai@spring8.or.jp, hiromura@spring8.or.jp, nmizuno@spring8.or.jp, kunio.hirata@riken.jp, kazuya@spring8.or.jp, y-nakamu@spring8.or.jp

Macromolecular crystallography (MX) has advanced as an essential structural analysis technique for modern life science research and drug discovery research with the development of excellent micro- focus beamlines, fast readout detectors, and smart crystal handling equipment.

At the micro-focus beamline BL32XU at SPring-8, we have been developing an automatic data col- lection system named ZOO [1, 2] so that all users can easily collect large amounts of diffraction data from high-difficulty samples. In addition, strategies with automated crystal positioning and radiation damage estimation can control data quality. As a result, the ZOO system has enhanced experimental efficiency and data quality, accelerated the accumulation of better data sets within the limited beam time, and opened the high-resolution structural analysis of challenging targets.

The ZOO system can also collect a few hundred complete data sets within a day. The capability also benefits high-throughput data collection of fragment-based drug design (FBDD) by examining many crystals in a complex with ligands. For this purpose, the beamline BL45XU was rebuilt to a fully automated high-throughput MX beamline with the ZOO system and the high-speed crystal handling system SPACE-II [3]. The expansion of developed technologies among SPring-8 beamlines eventually benefits all users. However, due to COVID-19 from 2020, many users could not visit the SPring-8 site, and more than 90% of the beamtime is now used for fully automated data collection at the BL45XU.

Simultaneously with the beamline development, we established the automated structure analysis pipeline NABE system and the ligand screening pipeline using an acoustic liquid handler ECHO for more efficient data collection and structure analysis.

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Fixed target system for biological research at PAL-XFEL

Author: Sehan Park[1] **Co-authors:** Jaehyun Park [2]; Jangwoo Kim [2]; SaeHwaN chun [2]; Ki Hyun NAM [3]; Intae Eom [2]

[1] *PAL-XFEL*

[2] *Pohang Accelerator Laboratory*

[3] *POSTECH*

Corresponding Authors: jaehyun.park@postech.ac.kr, saehwan.chun@postech.ac.kr, i.eom@postech.ac.kr, struc- tures@postech.ac.kr, jkpal@postech.ac.kr, sehan@postech.ac.kr

Serial Femtosecond Crystallography (SFX) offers several advantages in protein structure analysis, such as the ability to conduct experiments at room temperature with minimized radiation damage. However, SFX experiments using LCP (Lipidic Cubic Phase) suffer from stability issues in chemical reaction with carrier matrix and/or sample flow control. Recently, to overcome these problems, the fixed target technique based on raster scanning has been developed and utilized. In addition to avoiding liquid flow control and related laborious sample preparation process, this technique allows to determine protein structures with similar sample quantity for LCP injection technique under relatively simple sample treatment process. We have developed a unique 2D fixed target chip to perform SFX experiments1.The sample chip consists of a nylon mesh, thin polyimide films and metal frames. Micro-crystals are distributed on the nylon mesh that significantly reduce falling of crystals on the chip due to gravity. The thin polyimide films are attached both sides of the mesh to keep the fully hydrated condition. In addition, we have applied metal frames to hold the sample chip instead of plastic one to increase the efficiency during the successive chip exchanges. The material of the metal is magnetic 304 stainless steel. The 2-dimensional chip positions are controlled with the combination of SmarAct piezo-stages. The amount of required sample to prepare single chip is about 60 µl and the number of collectable images is about 130,000 at 50 µm step over 18 mm ranges, which will be sufficient data for determining a protein structure with single chip. I will introduce the novel 2D fixed target system including its structure and related operational features at PAL-XFEL in detail.

1 D. Lee *et al.*, *Sci. Rep.* **9**, 6971 (2019)

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Protein Production Sweden (PPS)

Authors: Maria Gourdon[1]; Wolfgang Knecht[2]

[1] *LP3, Lund University*

[2] *Protein Production Sweden (PPS), Lund node, Lund University*

Corresponding Authors: maria.gourdon@biol.lu.se, wolfgang.knecht@biol.lu.se

Protein Production Sweden (PPS; www.gu.se/pps) is a new national research infrastructure estab- lished in 2022 and focused on the production and purification of protein reagents for Swedish re- searchers, both from academia and commercial entities. Well-established protein production plat- forms from five universities (the University of Gothenburg (host), Karolinska Institutet, KTH Royal Institute of Technology, Lund University, and Umeå University) are collaborating to form the in- frastructure and offer expert competence in 4 geographically distributed nodes (Gothenburg, Lund, Stockholm, Umeå). Researchers across Sweden have access via a joint entry-point and have the possibility to get support based on their research needs throughout the whole process of protein production and purification, starting from project counselling and design to quality control, or for any single/multiple step(s) in the process.

PPS allows protein expression in 6 different expression systems (*E. coli*, *P. Pastoris*, Insect cells (BEVS), Plant cells, Mammalian Cells and Cell free expression). It also offers two Gateway modules aiming at producing speciality reagents for use in neutron scattering or X-ray crystallography.

PPS has the aim to be a broad state-of-the-art protein production infrastructure that delivers high quality protein reagents for all researchers in Sweden.

For more information, please see: www.gu.se/pps (pps@gu.se).

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Protein Characterization, Crystallization and Structure Determination at the Lund Protein Production Platform

Authors: Maria Gourdon[1]; Céleste Sele[1]; Wolfgang Knecht[2]

[1] *LP3, Lund University*

[2] *LP3, PPS, Lund University*

Corresponding Authors: wolfgang.knecht@biol.lu.se, maria.gourdon@biol.lu.se, celeste.sele@biol.lu.se

Lund Protein Production Platform (LP3, www.lu.se/lp3) is a cross-faculty expert center and user facility of Lund University (LU). As such, we provide services and make equipment available in the areas of recombinant protein production, crystallization, biophysical characterization, and structure determination. We also act as the LU node of a new national distributed research infrastructure for protein production: Protein Production Sweden (PPS, www.gu.se/pps) and we are one part of MAX IV FragMAX platform for X-ray aided fragment screening (https://www.maxiv.lu.se/fragmax/). In addition, we provide the laboratories for the biological part of the Deuteration and Macromolecular Crystallization (DEMAX, https://europeanspallationsource.se/science-support-systems/demax) plat- form of the European Spallation Source ERIC (ESS) and closely collaborates with DEMAX. At our facilities we have an attractive selection of automated equipment for crystallization experiments, among these the Dragonfly and Mosquito (SPT Labtech), for optimization screen preparations and nanoliter crystallization set ups, as well as Rigaku automatic imaging system, for monitoring experi- ments over time using both visible and UV light. We also have a range of analytical methods ready to evaluate the quality of your protein sample, or alternatively improve your sample's formulation for increased stability, including the Omnisec (Malvern Panalytical) (for measuring absolute molecular weight and sample composition) and the Prometheus (Nanotemper), for temperature stability evalu- ation. We can also offer harvesting and testing of your crystals at BioMAX (MAX IV), and assist in data processing and structure determination. Our aim is to give our users custom-made solutions to match their needs in order to help them move forward in their research.

For more information and access to protein characterization, crystallization and structure determination: https://www.lp3.lu.se/protein-crystallisation-and-characterisation (lp3@biol.lu.se).

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Investigating the structural and functional implications of PKM2 mutations in cancer

Author: Saurabh Upadhyay[1] [1]
IIT Delhi
Corresponding Author: saurabh807993@gmail.com

The PKM2 protein is a key player in cancer metabolism acting as a central node in tumor growth and progression. We aimed to study the effect of cancer patient-derived mutations in the PKM2 protein on its structural and functional properties. We deciphered the effect of the mutations on the overall structure of the protein using X-ray crystallography, which revealed subtle structural perturbations in the three dimensional structure of the protein. The structural alterations may play a role in the changes that we observe in the functional characteristics of the protein. The mutations modulate important substrate and ligand binding affinities of the PKM2 protein, particularly affect- ing its ability to bind to FBP and ADP. The mutations also impair the tetrameric oligomeric state of the protein, favoring a dimeric conformation, which is known to be beneficial for tumor survival. Moreover, the pyruvate kinase activity of the PKM2 enzyme was also hampered which could be a di- rect functional consequence of the biophysical and biochemical changes induced by the mutations. The C474S and R516C mutants were unresponsive to the FBP mediated change in the oligomeric state of PKM2, while L144P and P403A mutant proteins exhibited FBP mediated allosteric activation, as C474S and R516C mutations directly or indirectly impact the FBP binding site. Furthermore, we also explored the effect of an alkaline pH of cancer cells on the mutant proteins which decreased the kinetic efficiency of the enzymes, possibly by altering the chemistry of catalysis. In addition to this, we found that the PKM2 protein may also play a role in the beneficial effects of hyperthermic oncotherapy via functional disruption of the pyruvate kinase activity of PKM2 enzyme. Overall, our results suggest that cancer-related mutations in the PKM2 protein affect its function and structure, which may support its oncoprotein activity. This could lead to more aggressive forms of cancer in patients compared to those with the wild-type PKM2 enzyme.

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Investigating the structural and functional implications of PKM2 mutations in cancer

Author: Saurabh Upadhyay[1] [1] *IIT Delhi*
Corresponding Author: saurabh807993@gmail.com

The PKM2 protein is a key player in cancer metabolism acting as a central node in tumor growth and progression. We aimed to study the effect of cancer patient-derived mutations in the PKM2 protein on its structural and functional properties. We deciphered the effect of the mutations on the overall structure of the protein using X-ray crystallography, which revealed subtle structural perturbations in the three dimensional structure of the protein. The structural alterations may play a role in the changes that we observe in the functional characteristics of the protein. The mutations modulate important substrate and ligand binding affinities of the PKM2 protein, particularly affect- ing its ability to bind to FBP and ADP. The mutations also impair the tetrameric oligomeric state of the protein, favoring a dimeric conformation, which is known to be beneficial for tumor survival. Moreover, the pyruvate kinase activity of the PKM2 enzyme was also hampered which could be a di- rect functional consequence of the biophysical and biochemical changes induced by the mutations. The C474S and R516C mutants were unresponsive to the FBP mediated change in the oligomeric state of PKM2, while L144P and P403A mutant proteins exhibited FBP mediated allosteric activation, as C474S and R516C mutations directly or indirectly impact the FBP binding site. Furthermore, we also explored the effect of an alkaline pH of cancer cells on the mutant proteins which decreased the kinetic efficiency of the enzymes, possibly by altering the chemistry of catalysis. In addition to this, we found that the PKM2 protein may also play a role in the beneficial effects of hyperthermic oncotherapy via functional disruption of the pyruvate kinase activity of PKM2 enzyme. Overall, our results suggest that cancer-related mutations in the PKM2 protein affect its function and structure, which may support its oncoprotein activity. This could lead to more aggressive forms of cancer in patients compared to those with the wild-type PKM2 enzyme.

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Cryo Soft X-ray Tomography Beamline at Taiwan Photon Source

Primary author: Dr LIN, Zi-Jing (National Synchrotron Radiation Research Center)

Co-authors: Mr HSIEH, Chia-Chun (National Synchrotron Radiation Research Center); Dr HUA, Mo Da-Sang (National Synchrotron Radiation Research Center); Dr LAI, Lee-Jene (National Synchrotron Radiation Research Center); Dr LIN, Yi-Hung (National Synchrotron Radiation Research Center,); Dr SU, Yi-Jr (National Synchrotron Radiation Research Center)

Content

Visualize the subcellar structures within cells is important to understand biological phenomena in details for biomedical research. Microscopy plays and important role to fulfill this demand. Among all the microscopy techniques, transmission electron microscopy (TEM) and fluorescence microscopy are two major imaging modalities to explore the cellular structures. Fluorescence mi- croscopy can image in-vivo cell samples with nanometer resolution but only fluorescent-labelled structures can be observed. TEM allows to image the fine structures of organelles with high con- trast and extraordinary resolution of few nanometers. However, thickness of sample is limited because of low penetration depth of electron and sample preparation for TEM is relatively time-consuming.

Soft X-ray tomography (SXT) is a relatively new imaging tool in imaging entire cells. The imag- ing of SXT uses X-ray source with energy between 284eV and 543eV, which are the K absorption edges between carbon and oxygen. In this so called “water window” region, water is nearly trans- parent but carbon and nitrogen, the major constituents of biomolecules, are absorbing. Imaging in this range thus allows to obtain the high contrast carbon-based images of hydrated, intact cells in few micrometers thickness without the needs of time-consuming sample preparation procedures, such as labelling, embedding and sectioning. Due to this uniqueness, SXT can be an excellent complementary imaging technique for fluorescence microscopy and TEM. SXT beamline at Tai- wan Photon Source (TPS) in National Synchrotron Radiation Research (NSRRC) is designed with achievable resolution of 15–30 nm for 2D imaging and 50 nm for 3D imaging. SXT beamline is now partially open to public. Here, we report the current commissioning progress of SXT beamline. We also demonstrate the preliminary results of imaging cryo-fixed biological samples by using SXT.

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Three-dimensional cell imaging by cryo-soft X-ray tomography at Taiwan Photon Source

Primary author: Dr LAI, Lee-Jene (National Synchrotron Radiation Research Center)

Co-authors: Dr LIN, Zi-Jing (National Synchrotron Radiation Research Center); Mr HSIEH, Chi- a-Chun (National Synchrotron Radiation Research Center); Dr LIN, Yi-Hung (National Synchrotron Radiation Research Center); Dr HUA, Mo Da-Sang (National Synchrotron Radiation Research Cen- ter)

Content

Cryo-soft X-ray tomography (SXT) with full-field transmission microscopy is a new growing synchrotron- based technique, which is developed to image 3D complete frozen cells at Taiwan Photon Source TPS 24A beamline in National Synchrotron Radiation Research Center (NSRRC)[1]. To avoid the radiation damage, bio-samples have to be frozen in liquid ethane condition and transferred to be imaged by soft X-ray irradiation. We adopted an energy at 520 eV which is an energy in water window, energies between K-edge absorption of carbon (284 eV) and oxygen (543 eV), to image the cell sample, which could produce high absorption of carbon and ignore the oxygen absorption from the water to obtain the image in natural contrast. SXT is a complementary technique with fluorescence microscopy and electron microscopy to allow quickly 3D native cell image without the requirement of dye labelling and sample slicing. In TPS 24A, an off-line cryo-fluorescence microscopy was currently employed to obtain fluorescence image which could be correlated with the image from SXT to understand the functional and structural information on region of interest (ROI) of samples. Therefore, some biomedical studies can be conducted by SXT, such as mast cell degranulation, drug development and interaction of microorganism with host cells [2-4]. We will demonstrate 3D morphology of organelles in cells clearly, including mitochondria, lipid droplet and nuclear membrane from frozen cells in this meeting.

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Investigating depth-wise cell properties in articular cartilage via synchrotron phase-contrast microtomography

Author: Edvin Tobias Bokvist Wrammerfors[1]

Co-authors: Hector Dejea ; Maria Pierantoni [2]; Gustavo Orozco [2]; Martin Englund [3]; Hanna Isaksson [1]

[1] *Lund University*

[2] *Department of Biomedical Engineering, Lund University (LU), Lund, Sweden*

[3] *Clinical Epidemiology Unit, Orthopedics, Department of Clinical Sciences Lund, LU, Lund, Sweden*

Corresponding Authors: edvin_tobias.bokvist_wrammerfors@bme.lth.se, maria.pierantoni@bme.lth.se, hanna.isaksson@bme.lth.se hector.dejea@maxiv.lu.se, martin.englund@med.lu.se, gustavo.orozco@bme.lth.se

Introduction: Osteoarthritis (OA) is a debilitating joint disease affecting the middle-aged and elderly population, characterized by the progressive degeneration of cartilage and other joint tis- sues (1). Since the mechanisms are not fully understood, there is a need for non-destructive, high- resolution methods to identify structural changes in different disease stages. Phase-contrast syn- chrotron microtomography is a novel technique which enables high contrast in soft tissues such as cartilage and meniscus (2). Here we aim to investigate depth-wise cell properties in human cartilage, in order to understand the potential interplay between cell morphology change and tissue matrix degradation during OA progression.

Methods: We imaged ~4 mm diameter medial compartment human articular cartilage plugs (n=51, ages 18-84y), from donors without known knee OA (n=47) and total knee replacement (TKR) patients (n=4). Samples were imaged at the TOMCAT beamline (PSI, Switzerland) (2.7 µm voxel size, 21 keV, 40 cm propagation distance, 9 ms exposure time, 2000 projections) for an effective resolution of ~9 µm. Cells were segmented using an adaptive thresholding algorithm to determine depth-wise properties.

Results: Images clearly visualize cells in healthy samples as well as substantial fibrillation and cell morphology changes in TKR samples (Fig. 1). Qualitative analysis showed increased cell density and cell cluster size in deeper layers of cartilage.

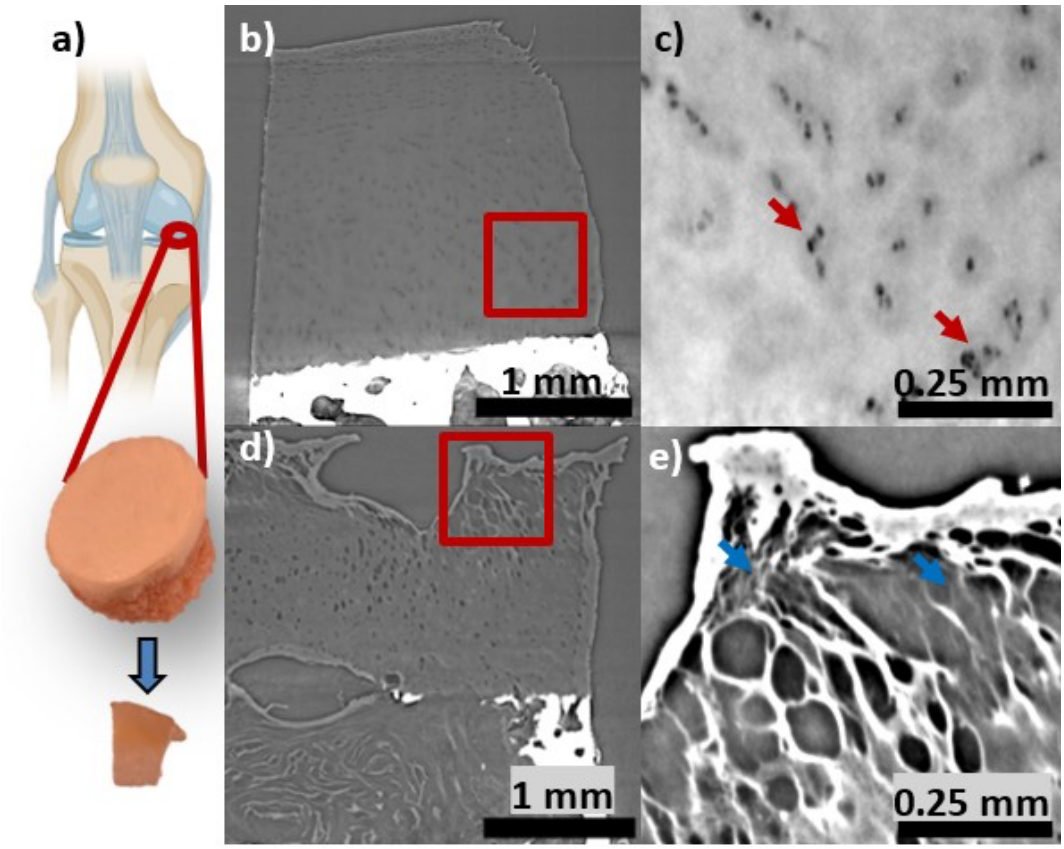

Figure 1: a) Schematic showing sample location in the medial compartment of the knee joint. b-c) Tomograph of a healthy articular cartilage sample, with enlarged region (c) clearly showing cells (red arrows). d-e) Tomograph of a degenerated sample, with enlarged region (e) clearly showing substantial fibrillation (blue arrows).

Figure 2: Figure 1: a) Schematic showing sample location in the medial compartment of the knee joint. b-c) Tomograph of a healthy articular cartilage sample, with enlarged region (c) clearly showing cells (red arrows). d-e) Tomograph of a degenerated sample, with enlarged region (e) clearly showing substantial fibrillation (blue arrows).

Discussion: Being a full 3D technique, synchrotron tomography reduces the intra-sample variance inherent in studying 2D-slices allows for more robust studies of cell properties and their connec- tion to donor age and disease state. Furthermore, this technique is non-destructive and does not require fixation or contrast agents, enabling in-situ experiments as well as combination with other techniques.

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The High Throughput Macromolecular Crystallography beamlineP11 at PETRA III

Authors: Guillaume Pompidor[1]; Spyros Chatziefthymiou[2]; Alexander Grebentsov[2]; Andrey Gruzinov[2]; Song Jial- ing [2]; Helena Taberman[2]; Hakanpää Johanna[2]

[1] *DESY*

[2] *Deutsches Elektronen-Synchrotron DESY, Photon Science*

Corresponding Author: guillaume.pompidor@desy.de

The P11 beamline at PETRA III (DESY, Hamburg) is a versatile instrument dedicated to High Through- put Macromolecular Crystallography 1, which provides variable focus sizes (from 200 x 200 to 5 x 10 µm2) in an energy range from 6 to 28 keV with a maximum of 1013 ph/s.

Since 2021, an EIGER2 X 16M detector is permanently installed at the beamline, which significantly reduced the data collection time. The beamline is equipped with an automated sample changer (de- war capacity of 23 unipucks ie 368 samples) with a "mounting and un-mounting" cycle of 36s. The total time per sample (including sample changing and full data collection) is less than 2 minutes.

In 2023, we will change our data acquisition software to MXCuBE. The integration to ISPyB, for tracking shipments, importing the sample details to MXCuBE and data archiving, is under progress. The establishment of parallel autoprocessing pipelines in addition to the current one based on XD- SAPP [2] and the implementation of strategy calculation including dose estimation are on going. These software developments are synchronizing P11 with the EMBL PETRA III beamlines for MX (P13 and P14) for the future foundation of a uniform structural biology village at PETRA IV.

Serial crystallography can be performed at P11 either with standard mounts or with the TapeDrive [3] developed at the CFEL, specially suited for time resolved experiments using the mix and dif- fuse method. Real-time processing of serial data using CrystFEL [4] has been recently implemented. The pipeline can successfully perform peak searching, indexation and integration in real time at the maximum data acquisition speed (133Hz).

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Multimodal and multiresolution X-ray studies of nanoparticle pathways in plants.

Authors: Emil Visby Østergaard[1]; Francesca Siracusa[2]; Rajmund Mokso[3]

[1] *Technical university of Denmark*

[2] *DTU*

[3] *Max IV Laboratory, Lund University*

Corresponding Authors: fransi@dtu.dk, emilv@dtu.dk, rajmund.mokso@maxiv.lu.se

Mineral nutrients are taken up by plants through their root network, therefore if additional nutrient is required, this is today applied to the soil in vast quantities. From this, the crops take up only a minor fraction 1. We are researching the possibility to apply nutrients through foliar fertilization, developing nanoparticles (NPs) as vessels to deliver the fertilizer through the leaf into the plant. Our aim is to complement the traditional analytical methods such as laser ablation to determine the con- tent of a certain element by X-ray based methods. We develop protocols for how to apply X-ray micro and nano-tomography, SAXS, and X-ray fluorescence to probe live and freeze-dried plants. Recent observations suggest that mineral nutrients (P, Mn, and Zn) delivered as nanoparticles (NPs) can enter the plant through the leaves and be delivered to the target location inside the plant. How- ever, several instances of the NPs pathways rely on hypotheses rather than experimental evidence. For example, the role of stomata is significant, yet remains poorly understood [2]. Once inside the leaf, NPs must be able to enter the cells, and ideally also the vascular tissues (phloem), where trans- port to other parts of the plant occurs.

We have employed multiscale X-ray tomography using both lab and synchrotron-based radiation to visualize NP agglomeration and translocation as a function of both time and location in plant. In µCT (0.385 - 3 µm voxel size) MnO NPs applied to soy plants are visible as regions of contrast (seen as red in figure 1 a)) shortly after application and potential agglomerates in the vascular tissue a cer- tain time after application. nCT (50 nm voxel size) measurements show high quality visualizations of the cellular structure of chloroplasts in the leaf and both xylem and phloem in the vascular tissue. Agglomerates (>0.5 µm) from NP solution drying on the leaf surface are visible, but no significant particle agglomerates are visible inside the plant structure. To support these measurements, we aim to do multimodal experiments employing SAXS and XRF in combination with tomography to obtain knowledge of NP behavior (transport, clustering, bioavailability, and dissolution) while having the 3D visualization capability of X-ray CT.

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Harnessing low background data collection at the nano-focus beam- line, VMXm, for structure determination of membrane proteins from microcrystals.

Author: Adam Crawshaw[1]

Co-authors: Anna Warren [1]; Andrew Quigley [1]; Jose Trincao [1]; Gwyndaf Evans [1]

[1] *Diamond Light Source*

Corresponding Author: adam.crawshaw@diamond.ac.uk

Structure solution of membrane bound proteins remains a significant challenge. These targets, such as potentially druggable GPCRs, represent ~3% of the X-ray diffraction structures in the PDB. Crys- tallisation often requires the use of lipid cubic phase (LCP) to maintain the hydrophobic environment, protein stability and promote crystallisation. Membrane protein LCP crystals are generally smaller and more fragile than other protein crystals. As such, a microfocus beamline is often needed for data collection.

The Versatile Macromolecular Crystallography beamline (VMXm) provides a stable X-ray beam of 0.4-10 µm x 1.5-5 µm (V x H), an in-vacuum sample environment and integrated scanning electron microscope to enable high quality, low background, single crystal data collection from crystals mea- suring 0.5-10 µm. Significant effort is made to ensure that the sample is mounted in such a way as to minimise additional X-ray scatter. Together the beamline apparatus and standard sample mount contribute minimal background noise.

However, LCP generates a significant amount of X-ray scatter, so it is essential to remove as much of the LCP as possible. This is challenging for LCP derived microcrystals, where providing a suitable, low noise sample mount is difficult while also minimising the amount of LCP material all without damaging the fragile microcrystals. Some of these challenges have already been addressed for LCP sample preparation for electron diffraction 1.

We will outline the sample preparation process for LCP derived membrane protein crystals for VMXm and examples of its deployment at the beamline. We will also demonstrate how the use of VMXm and these sample preparation methods enable data collection from previously very difficult to access membrane protein samples.

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In-situ analysis of the protein adsorption on solid-supported lipid layers

Author: Jaqueline Savelkouls[1]

Co-authors: Christian Sternemann [1]; Michael Paulus [1]; Mike Moron [1]; Susanne Dogan-Surmeier [1]

[1] *Technische Universität Dortmund*

Corresponding Author: jaqueline.savelkouls@tu-dortmund.de

Biochemical processes at lipid membranes are essential for all living organisms and can be influenced by the interaction of proteins with lipid interfaces [1-2]. Hence, the effects of protein adsorption at such surfaces are important for understanding the organisms' biochemistry, for current issues in the pharmaceutical industry and for bio- and nanotechnology [3-5].

We studied the adsorption of the proteins ferritin and apoferritin at a hydrophobic solid/liquid in- terface with X-ray reflectometry at beamline BL9 of the synchrotron radiation source DELTA (Dort- mund, Germany) [6,7] at an incident photon energy of 27 keV. The model system is a stable phospho- lipid bilayer of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) applied by spin coating on a silicon single crystal wafer [8].

To study the adsorption processes under the influence of pH and protein concentration at room temperature, the lipid layers are applied in an aqueous environment in a dedicated polytetrafluo- roethylene sample cell.

Measurements on a DMPC layer with apoferritin show an increase in roughness, electron density and layer thickness of the head groups adopted to the Si/SiO2 surface. This suggests that the protein accumulates in this area. With higher protein concentration, structural changes in the whole lipid system can be recognized. Especially the roughness of the lipid layer increases.

A decrease in pH also causes a change in roughness and electron density. At low pH values, the layer thickness around the head groups at the SiO2 interface grows, which is explained by the con- formational change of the protein [9]. In comparison, the alkyl chains maintain their length.

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Generating predicted models with CCP4

Author: Ville Uski[1]

Co-authors: Ronan Keegan [2]; Charles Ballard [2]; Eugene Krissinel [3]; Grzegorz Chojnowski [4]

[1] *Science and Technology Facilities Council*

[2] *CCP4*

[3] *Science and Technology Facilities Council UK*

[4] *EMBL Hamburg* **Corresponding Authors:** ronan.keegan@stfc.ac.uk, ville.uski@stfc.ac.uk, eugene.krissinel@stfc.ac.uk, charles.ballard@stfc.ac.uk, gchojnowski@embl-hamburg.de

We discuss recent work on making use of deep-learning-based protein structure prediction programs with the CCP4 Software Suite. These programs include AlphaFold2 1 and OpenFold [2], which use multiple-sequence alignments as input, and ESM-Fold [3], which uses embeddings from protein Language Models. We have developed an adapter script that allows running these programs via a unified interface. The script is distributed with CCP4 and has been integrated in the structure- prediction task of CCP4 Cloud, a front-end of the Suite. We have installed a local mirror of the AFDB database that is used in CCP4 Cloud to find and prepare molecular-replacement models with MrParse [4]. We discuss these developments and related work.

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BioMAX: Trends and developments at a 4th generation Macro- molecular Crystallography Beamline

Author: Ana Gonzalez[1]

Co-authors: Swati Aggarwal ; Oskar Aurelius [2]; Monika Bjelčić ; Manoop Chenchiliyan ; Mikel Eguiraun ; Aaron Finke ; Ishkhan Gorgisyan ; Tobias Krojer [3]; Elmir Jagudin ; Mirko Milas ; Julio Lidon-Simon [4]; Jie Nan [5]; Marjolein Thunnissen [6]; Meghdad Yazdi ; Uwe Mueller [7]; Thomas Ursby [4]

[1] *MAX IV*[2] *MX*[3] *BioMAX*[4] *MAX IV Laboratory*[5] *MX-group*[6] *Lund University*[7] *HZB*

Corresponding Authors: mikel.eguiraun@maxiv.lu.se, elmir.jagudin@maxiv.lu.se, julio.lidon-simon@maxiv.lu.se, ishkhan.gorgisyan@maxiv.lu.se, aaron.finke@maxiv.lu.se, uwe.mueller@helmholtz-berlin.de, jie.nan@maxiv.lu.se, mirko.milas@maxiv.lu.se, marjolein.thunnissen@maxiv.lu.se, thomas.ursby@maxiv.lu.se, ana.gonzalez@maxiv.lu.se, tobias.krojer@maxiv.lu.se, manoop.chenchiliyan@maxiv.lu.se, monika.bjelcic@maxiv.lu.se, meghdad.yazdi@maxiv.lu.se, oskar.aurelius@maxiv.lu.se, swati.aggarwal@maxiv.lu.se

The MAX IV laboratory hosts the first 4th generation storage rings. Currently MAX IV operates two Macromolecular Crystallography (MX) beamlines, BioMAX, and MicroMAX. The BioMAX beamline became operational in 2017. The beam can be focused to 20 x 5 µm² FWHM with a pho- ton flux of about 5x10¹² photons/s. Changes of energy between 6 and 24 keV and beam defocusing up to 100 x 100 µm² are automated. The experimental hutch is equipped with a MD3 microdiffractometer, an IRELEC Isara sample changer and a 16M Eiger detector. Data collection at cryotemperatures can be done fully remotely. BioMAX is a highly versatile beamline, capable of handling a wide array of experimental techniques from conventional oscillation experi- ments, data collection from small crystals and unit cell, experimental phasing as well as to the state of the art technique of synchrotron serial crystallography (SSX).1

Current priorities at BioMAX are influenced by the current trends in Structural Biology, notably the increasing use of Cryo-EM, particularly for large complexes and membrane proteins, the advent of AlphaFold for prediction of accurate models of protein structures and the interest in the study of pro- tein dynamics at room temperature. While maintaining user friendly capacities for de novo phasing, like high speed energy scans and automated SAD and MAD data collection, efforts have concentrated in the development of fully automated “unattended” data collection to handle an increasing demand for fragment based drug discovery projects enabled by the development of the FragMAX platform[2] and industrial beamtime, implementation of in-situ data collection and remote data collection for room temperature experiments, and developing and testing sample delivery instruments and data handling procedures for the new sister beamline MicroMAX a cutting edge beamline specialized in microfocus applications and time-resolved experiments.

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Moorhen: a web molecular graphics program

Author: Filomeno Sanchez[1]

Co-authors: Stuart McNicholas [1]; Paul Emsley [2]; Martin Noble [3]; Kevin Cowtan [1]

[1] *University of York*

[2] *MRC Laboratory of Molecular Biology*

[3] *Newcastle University*

Corresponding Authors: filomeno.sanchezrodriguez@york.ac.uk, martin.noble@newcastle.ac.uk, kevin.cowtan@york.ac.uk, stuart.mcnicholas@york.ac.uk, pemsley@mrc-lmb.cam.ac.uk

Here we present Moorhen, a next-generation client-side web-based application for the visualisation and manipulation of molecules in structure determination and analysis.

Recent developments in the field of cloud-based computing have enabled a rapid increase in the range of crystallographic tools available on the web, which crystallographers can use without the need for installing software locally. This provides great opportunities in making scientific software more available in teaching contexts and to previously underrepresented communities. However, several challenges remain untackled, in particular those in the field of molecular graphics. There is the need to provide users with web-based tools for the visualisation and manipulation of complex molecular structures together with their associated experimental data.

Moorhen is a new web-based application which provides access to a large number of functionalities developed as part of the Coot model building software and exposed in the libcoot API via an interac- tive user-friendly graphical interface built with React. Thus, Moorhen is the first generally available tool of its kind offering tools for interactive model editing and model refinement on the web. All of Moorhen's refinement and visualisation calculations are performed "in browser", which means users have the ability to access these model editing tools with near-native performance without the drawback of installing any additional software in their machines. Moorhen has been made available as a stand-alone web-app at moorhen.org, and also through the CCP4 Cloud graphical user inter- face (GUI). Additionally, there are plans in place to extend its availability through the CCP4i2 and CCPEM GUIs.

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PReSTO: Structural Biology Software in a High Performance Com- pute Environment

Authors: Marcos Acebes[1]; Alexandra Ahlner[2]; Oskar Aurelius[3]; Aaron Finke[None]; Ana Gonzalez[4]; Karl Hörnell[5]; Christian Luckey[5]; Zdenek Matej[None]; Martin Moche[6]; Jie Nan[7]; Torben Rasmussen[8]; Anders Sjöström[1]; Maria Sunnerhagen[None]; Sebastian Thorarensen[5]

[1] *Lund University*

[2] *Linköping University*

[3] *MX*

[4] *MAX IV*

[5] *National Supercomputing Centre*

[6] *Karolinska Institutet* [7] *MX-group*

[8] *National Supercomputer Centre*

Corresponding Authors: aaron.finke@maxiv.lu.se, alexandra.ahlner@liu.se, zdenek.matej@maxiv.lu.se, maria.sunnerhagen@liu.se, marcos.acebes@lunarc.lu.se, torbenr@nsc.liu.se, jie.nan@maxiv.lu.se, oskar.aurelius@maxiv.lu.se, ana.gonzalez@maxiv.lu.se, sebth@nsc.liu.se, anders.sjostrom@lunarc.lu.se, karho@nsc.liu.se, martin.moche@ki.se

PReSTO is a software stack for integrated structural biology adapted to high performance comput- ing resources at the Swedish National Infrastructure for Computing (SNIC) and the local MAX IV compute cluster. Our aim is to support integrative structural biologists evaluate their data from macromolecular X-ray crystallography (MX), X-ray free electron lasers (XFEL), nuclear magnetic resonance spectroscopy (NMR), cryo-electron microscopy (cryo-EM), neutron scattering (NMX), and small-angle X-ray scattering (SAXS). The purpose of PReSTO is to provide users with convenient access to useful software already set up and optimized for powerful supercomputing platforms. The PReSTO team supports software updates and bugs and assists users by facilitating access to the various platforms we support.

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MicroMAX: a beamline for dynamic macromolecular crystallog- raphy at the MAX IV Laboratory

Author: Oskar Aurelius[1]

Co-authors: Mirko Milas [1]; Manoop Chenchiliyan [1]; Meghdad Yazdi-Rizi [1]; Jie Nan [1]; Ishkhan Gorgisyan [1]; Monika Bjelčić [1]; Staffan Benedictsson [1]; Linus Roslund [1]; Elmir Jagudin [1]; Mikel Eguiraun [1]; Alberto Nardella [1]; Aaron Finke [1]; Tobias Krojer [1]; Ana Gonzalez [1]; Thomas Ursby [1]

[1] *MAX IV Laboratory*

Corresponding Authors: alberto.nardella@maxiv.lu.se, mikel.eguiraun@maxiv.lu.se, elmir.jagudin@maxiv.lu.se, linus.roslund@maxiv.lu.se, tobias.krojer@maxiv.lu.se, aaron.finke@maxiv.lu.se, staffan.benedictsson@maxiv.lu.se, ana.gonzalez@maxiv.lu.se, monika.bjelcic@maxiv.lu.se, thomas.ursby@maxiv.lu.se, mirko.milas@maxiv.lu.se, meghdad.yazdi@maxiv.lu.se, manoop.chenchiliyan@maxiv.lu.se, jie.nan@maxiv.lu.se, ishkhan.gorgisyan@maxiv.lu.se, oskar.aurelius@maxiv.lu.se

The second macromolecular crystallography (MX) beamline at the MAX IV Laboratory, MicroMAX, collected its first protein dataset at the end of 2022. With the BioMAX beamline having established a robust framework for high-throughput crystallography, MicroMAX will aim to complement these abilities in the form of supporting experiments needing a higher flux density, more custom sample environments or non-standard experiment control, and to provide further tools to study dynam- ics.

MicroMAX will deliver experimental flexibility by being able to operate in wider bandwidth modes (up to ~ 1% ΔE/E) with maximised flux and exposure times down to 10s of μs, as well as different focusing schemes (compound refractive lenses, or Kirkpatrick–Baez mirrors). To benefit from the high flux density of the beamline, a nanosecond pump laser system from EKSPLA, covering the wavelength range of 210 – 2600 nm, for pump-probe experiments has been installed. An additional aid for studying dynamics, and providing options to mitigate radiation damage spread to neighbour- ing samples, will be a magnetically levitating chopper system from Celeroton, that can operate up to a pulse rate of 2.2 kHz. This setup will later be complemented with a gain-switching integrating JUNGFRAU detector from the Paul Scherrer Institute.

Capabilities also include support for rotational data collection at narrow-bandwidth, using a high- precision MD3-UP diffractometer from Arinax, an ISARA robotic sample changer from IRELEC and an EIGER2 X 9M CdTe detector from DECTRIS. In narrow-bandwidth mode, MicroMAX will be able to operate up to 25 keV photon energy and benefit from a maintained high quantum efficiency of the CdTe detector at these energies.

General user operation of the beamline will start in the third quarter of 2023 and MicroMAX looks forward to welcoming users for studying dynamics, high-throughput rotation or serial data collec- tion at different temperatures and for other types of experiments that could benefit from its highly focused bright beam.

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Stabilization of mRNA lipid nanoparticles

Author: Sibel Uzuncayir[1] [1]
Lund University
Corresponding Author: sibel.uzuncayir@med.lu.se

The Covid-19 pandemic resulted in the release of the new class of vaccine products- the mRNA vaccines. These consist of synthetic mRNA strands packed in lipid nano particle (LNP) that deliver the mRNA to the cells, initiate antigen production and lastly result in immune protection. The function of mRNA in vivo depends on effective, safe and stable delivery to allow cellular uptake and RNA release.

One of the main disadvantages of mRNA vaccines is that they need to be stored at ultra-low temper- atures, hampering the world-wide distribution during the pandemic. To overcome this hurdle the characterization of the structure-activity relationship of mRNA LNPs formation is crucial for fully understanding their mechanism and in order to improve their stability. LNPs are multicomponent systems consisting of a shell and a core. The shell consists of PEG, a helper lipid such as DSPC and Cholesterol, whereas the core mainly consists of an ionizable lipid and mRNA. In particular, the interaction of mRNA, water and ionized lipids in the core of LNP seems to play a major role in degradation and understanding these processes will aid to optimize mRNA LNP design in the future.

Here, we determine structural features such as changes in morphology and degradation processes of mRNA LNP in different settings and with different lipid compositions by combining cryo-electron microscopy, SAXS, CV-SANS with LNP standard assays (DLS, Ribogreen, RNA integrity and protein expression) to gain insight in their formation kinetics and investigate the cause of their instability during storage.

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Sample identification using RFID in macromolecular crystallography beamlines at the Photon Factory

Authors: Masahiko Hiraki[1]; Naohiro Matsugaki[2]; Yusuke Yamada[2]; Masahide Hikita[3]; Toshiya Senda[2]

[1] *Mechanical Engineering Center, KEK*

[2] *Photon Factory, KEK*

[3] *High Energy Accelerator Research Organization*

Corresponding Authors: masahide.hikita@kek.jp, masahiko.hiraki@kek.jp

At the Photon Factory macromolecular crystallography beamlines, sample exchange system PAM and PAM-HC have been installed and operated by the Structural Biology Research Centre. Most of the samples are brought in by Uni-puck, and information of the samples in the Uni-puck is described in the sample list file corresponding to each Uni-puck. The Uni-puck is automatically recognized by the sample exchange system by reading the 2D barcode on the pin placed in a specific position of the Uni-puck. However, since 2D barcode sometimes have reading errors and reading all barcodes is time-consuming, we are considering using RFID tags to identify samples. We have successfully read RFID tags embedded in Uni-puck in liquid nitrogen via an L-shaped adapter, but long-term stable operation has not yet been achieved. Therefore, we are proceeding with a two-step method of sample identification. First, before the L-shaped adapter containing the Uni-pucks is placed in the liquid nitrogen Dewar, the RFID tags of the L-shaped adapter and up to four Uni-pucks are read and recorded. Next, only the RFID tags of the L-shaped adapters are read in liquid nitrogen Dewar to determine the location of all Uni-pucks. We have tested each of the steps and so far have confirmed the usefulness of the two-step reading.

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Background Impact on 3D CXDI reconstruction of single particles.

Authors: August Wollter[1]; Tomas Ekeberg[1]

[1] *Department of Cell and Molecular Biology, Uppsala University*

Corresponding Authors: tomas.ekeberg@icm.uu.se, august.wollter@icm.uu.se

Coherent X-ray diffractive imaging (CXDI) of single particles at X-ray free-electron lasers (XFEL) is a method to determine structure. Single particles are injected sequentially at unknown, random orien- tations into the intense XFEL beam and noisy diffraction patterns are measured, known as diffraction before destruction.1 We orient these patterns using the expand, maximize and compress (EMC) al- gorithm, resulting in a three-dimensional diffraction intensity map.[2] The goal of the method is to reconstruct the structure of the sample, but for this we also need to recover the phases which is possible by iteratively applying constraints in real space, a positive density in a restricted volume, and fourier space where we constrain the intensities using our result from EMC.

The samples are injected and aerosolized by electrospray and launched into the XFEL beam, but the carrier gas contributes to the diffraction patterns as background. [3] We want to help plan experiments to see if they are feasible given expected signal and background levels with regards to EMC and phase retrieval.

To test this we used background measurements from an electrospray injector at the European XFEL combined with simulated signal diffraction patterns of phytochrome. We tested the performance of the EMC algorithm with various amounts of background and signal photons in the diffraction patterns. After subtracting the background of the final models we could perform phase retrieval, solving the structure for many of the cases. Finally we could compare the results from the different combinations for EMC and phase retrieval.

We found by running EMC multiple times for the same combinations of signal and background that successful EMC convergence is insensitive to the random initial conditions. Using the phase retrieval transfer function (PRTF) [4] method of calculating the resolution of the reconstruction we can see how the resolution deteriorates as we increase the background.

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New Kid on the Beam: adding asymmetrical-flow field-flow fractionation (Af4) to tackle polydispersity

Authors: Melissa Graewert[1]; Clement Blanchet[1]; Peter Langguth[2]; Heinrich Haas[3]; Christoph Wilhelmy[2]; Bastian Kolb[2]; Jens Schumacher[None]; Tijanan Bacic[None]; Florian Meier[None]; Roland Drexel[None]; Dmitri Svergun[None]

[1] *EMBL Hamburg*

[2] *Uni Mainz*

[3] *Uni Mainz*

Corresponding Authors: clement.blanchet@embl-hamburg.de, melissa.graewert@embl-hamburg.de

Over the past years, it has been clearly demonstrated that incorporating size-exclusion chromatog- raphy (SEC) directly before the collection of SAXS data is very
beneficial as it ensures sample homogeneity. However, not all biological systems can be subjected to SEC without any drawback. Sample dilution, risk of sample
disruption through interaction with the column's stationary phase, and limitation in the choice of buffer and additives can often diminish the success of employing
SEC. For these samples, we propose using a different online separation approach – Asymmetrical Flow Field-Flow-Fractionation (AF4). Here, the separation

takes place in a thin liquid film and thereby prevents unwanted interaction effects. We recently were able to demonstrate the successful use of the removal of free mRNA from lipoplex formula- tions (LPX). Furthermore, important quantitative parameters could be retrieved for the separated particles.
Currently, we are installing an AF4 set-up at P12 and here, I discuss its potential benefits for a wide range of applications. This project is financed through the German BMBF (grant 05K22UM3).

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Structural insight into the synergistic activities of NADase and SLO in the pathogenesis of Group A Streptococcus infection

Author: Shu-Ying Wang[1]

Co-authors: Wei-Jiunn Tsai [2]; Michal Hammel [3]; U-Ser Jeng [4]

[1] *Department of Microbiology and Immunology, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

[2] *Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan*

[3] *Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, CA, USA*

[4] *National Synchrotron Radiation Research Center, Hsinchu Science Park, Hsinchu, Taiwan*

Corresponding Author: sswang23@mail.ncku.edu.tw

Group A Streptococcus (GAS) is a strict human pathogen renowned for its highly aggressive destruc- tion of host tissues that can lead to life-threatening diseases including streptococcal toxic shock syn- drome and necrotizing fasciitis. GAS possesses a unique pathogenic trait to enhance its virulence by utilizing the synergism of two secreted toxins, Streptolysin O (SLO) and NAD+-glycohydrolase (NADase). How NADase and SLO assemble into a complex to synergistically promote intracellu- lar survival and resistance to innate immunity within human cells is a long-standing question. By combining X-ray crystallography and small-angle X-ray scattering (SAXS), we decipher the first structure of NADase/SLO complex and reveal the dynamic nature of the complex in solution. The functionally-relevant conformations of the NADase/SLO complex revealed by SAXS suggest the dy- namic interplay between SLO and NADase is fundamental to the functioning of the complex. More- over, the introduction of a complex-disruptive mutation in GAS genome results in decreased resis- tance to phagocytic killing in vitro. Furthermore, mice infected with GAS mutant harboring the disruptive mutation displayed reduced skin lesions. This work delivers the structure-functional re- lationship of the NADase/SLO complex and pinpoints the key interacting residues that are central to the coordinated actions of NADase and SLO in the pathogenesis of GAS infection.

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Bone Regeneration in Alveolar Bone Biopsies as Retained in a Trephine Bur Using Synchrotron Radiation

Authors: Yong-Gun Kim[1]; Jae-Hong Lim[2]

[1] *School of Dentistry, Kyungpook National University*

[2] *4 Pohang Accelerator Laboratory, POSTECH, Pohang, Gyeongbuk, Korea*

Corresponding Author: periokyg@knu.ac.kr

Objectives: Trephine bur is the most common and effective instrument used in the biopsy of bone tissue to evaluate the results of bone regeneration procedures. Trephine bur can be mounted on the rotating instrument to obtain the regenerated tissue in a cylindrical shape. However, in the process of separating the new bone tissue from the trephine bur after tissue harvest, a physical force may be applied to the new bone tissue, which may result in deformation of the new bone tissue. We have developed a 3D assessment technique using synchrotron radiation X-ray μCT (SR-μCT) that does not require sample ejection from the TB.

Material and methods: Specimens were collected from six participants after 5-28 months of unevent- ful healing from an alveolar bone regenerative surgery. Specimens were kept in a fixing solution

after biopsy and until SR-μCT scan using a 50-keV monochromatic X-ray beam. Edge enhancement by the phase contrast effect of SR-μCT was utilized to improve imaging sensitivity. Images were re- constructed using a phase retrieval algorithm of simultaneous phase-and-amplitude retrieval (SPAR) and segmented for 3D visualization and quantification using the water-shedding algorithm.

Results: We successfully visualized the TB's interior through SRuCT related reconstructed images without removing TB. And we performed histomorphometry about three composition of newly formed bone (NFB), non-mineralized tissues (NMT) and residual bone graft material (RBG). Quan- tifications for characterizing individual bone regeneration revealed varying degrees of bone regen- eration among the specimens tested.

Conclusions: This study could facilitate a study of poor bone regeneration by ensuring a three- dimensional non-destructive examination of the regenerated bone tissue.

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Insights into the structure and function of membrane proteins from anomalous scattering of light atoms

Author: Ramona Duman[1]

Co-authors: Kamel El Omari [2]; Christian Orr [2]; Vitaliy Mykhaylyk [2]; Armin Wagner [2]

[1] *Diamond Light Source, UK*

[2] *Diamond Light Source*

Corresponding Authors: kamel.el-omari@diamond.ac.uk, vitaliy.mykhaylyk@diamond.ac.uk, armin.wagner@diamond.ac.uk, christian.orr@diamond.ac.uk, ramona.duman@diamond.ac.uk

More than a third of all proteins contain metal ions 1 with crucial roles in stabilizing structure or enabling a specific function. Identifying metal ion-binding sites is important for understanding the biological functions of proteins and further helps in designing potent therapeutics.

Membrane proteins have important roles to play in shuttling a variety of ligands and ions across lipid bilayers. The anomalous scattering properties of these ions can be harnessed to confirm their identity and location, to elucidate the mechanism of action of membrane proteins and to perform structure determination by experimental phasing. However, experimentally identifying and locating metal ions, such as calcium and potassium, in protein structures, can be challenging, since their X- ray absorption edges are inaccessible to most beamlines.

The unique wavelength range of the macromolecular crystallography beamline I23 at Diamond Light Source [2] allows identification and location of metal ions and lighter atoms of biological relevance (Ca, K, S, P and Cl) using X-ray anomalous scattering analysis. In a typical experiment, anomalous datasets are collected at two wavelengths, above and below the ion or element absorption edge, and then processed to calculate phased anomalous difference Fourier maps. The difference in anomalous peak heights between these two datasets allows the direct identification and visualisation of the ion in the protein structure.

The efficacy of this technique will be demonstrated using studies on membrane proteins investigating the gating mechanism of transporters, ion occupancy status through channels and multiple ion iden- tification. Examples of experimental phasing of membrane proteins using sulfur single-wavelength anomalous dispersion will also be highlighted.

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