Imaging CoE XFEL Serial Crystallography Workshop

19 May 2019

08:30	-	Coffee/tea	Duration	Registration – Level 2, Room 209
09:00	-	Connie Darmanin Brian Abbey *La Trobe University*	15 mins	Introduction/Welcome
09:15	-	Andrew Peele *ANSTO*	20 mins 5 mins Q&A	Crystallography at the Australian Synchrotron, ANSTO: status and plans
09:40	-	Henry Chapman (keynote speaker) *CFEL*	30 mins 5 mins Q&A	Serial crystallography and diffraction with X-ray FELs
10:15	-	Alexandra Ros *Arizona State University*	20 mins 5 mins Q&A	Microfluidic Tools for Serial Crystallography
10:40	-	Robert Feidenhans'l *EuXFEL*	20 mins 5 mins Q&A	European XFEL - First Results After 1.5 Years of Operation
11:05	-	Harry Quiney *Melbourne University*	10 mins 5 mins Q&A	Plasma dynamics in XFEL pump-probe experiments
11:20		Peter Berntsen *La Trobe University*	10 mins 5 mins Q&A	Serial Crystallography at the Australian Synchrotron
11:35	-	Lunch break – Level 2 Foyer 2.4		
12:15	-	**Bus departs from Melbourne Convention Centre**		

Workshop Venue
Level 2, Room 209
Melbourne Convention and Exhibition Centre
1 Clarendon Centre Pl, South Wharf,
VIC 3006, Australia

Schedule at the Australian Synchrotron

13:05 to 13:30	-	**Group A** Australian Synchrotron tour (Alan Ribodi & Rachel Williamson, ANSTO)	**Group B** Lipidico demonstration at the MX2 beamline (Peter Berntsen, LTU)	**Group C** Hit Finding Tutorial (Marjan Hadian-Jazi, LTU)	**Group D** Data analysis tutorial (Jun Aishima, ANSTO)
13:35 to 14:00	-	Lipidico demonstration at the MX2 beamline (Peter Berntsen, LTU)	Hit Finding Tutorial (Marjan Hadian-Jazi, LTU)	Data analysis tutorial (Jun Aishima, ANSTO)	Australian Synchrotron tour (Alan Ribodi & Rachel Williamson, ANSTO)
14:05 to 13:30	-	Hit Finding Tutorial (Marjan Hadian-Jazi, LTU)	Data analysis tutorial (Jun Aishima, ANSTO)	Australian Synchrotron tour (Alan Ribodi & Rachel Williamson, ANSTO)	Lipidico demonstration at the MX2 beamline (Peter Berntsen, LTU)
14:35 to 15:00	-	Data analysis tutorial (Jun Aishima, ANSTO)	Australian Synchrotron tour (Alan Ribodi & Rachel Williamson, ANSTO)	Lipidico demonstration at the MX2 beamline (Peter Berntsen, LTU)	Hit Finding Tutorial (Marjan Hadian-Jazi, LTU)
15:10	-	**Bus departs from AS**			
16:00	-	Return to Melbourne Convention Centre for further round table discussion and drinks – To meet at the IPAC Student Poster Session in the Main Foyer			

Serial Crystallography Data Analysis Tutorial

(Marjan Hadian Jazi - m.hadianjazi@latrobe.edu.au and Jun Aishima - juna@ansto.gov.au)

The recent development of serial crystallography at Macromolecular crystallography (MX) beamlines at synchrotrons (such as MX2) produce crystallographic datasets of ever-increasing volume from randomly orientated crystals. Data collected from these types of experiments use software pipelines assembled by combining processing software packages. We will demonstrate the individual steps of processing data from a serial crystallography MX experiment from the Lipidico apparatus using diffraction data of real biological crystal samples collected at the MX2 beamline with particular emphasis on the range of parameters that need to be adjusted at each step and their consequences on downstream results. Finally, we will discuss how the currently available tools for processing serial crystallography data can be used to create an automatic pipeline to make possible user experiments with rapid experiment feedback.

Lipidico demonstration: Show and Go at MX2

(Peter Berntsen - p.berntsen@latrobe.edu.au)

A serial millisecond crystallography (SMX) facility has recently been implemented at the Macromolecular crystallography beamline, MX2 at the Australian Synchrotron. The setup utilizes a combination of an EIGER X 16M detector system and an in-house developed high-viscocity injector, `Lipidico'. Lipidico uses a syringe needle to extrude the microcrystal-containing viscous media and is compatible with commercially available syringes. The combination of sample delivery via protein crystals suspended in a viscous mixture and a millisecond frame rate detector, enables high-throughput serial crystallography at the Australian Synchrotron. A hit-finding algorithm, based on the principles of ‘robust-statistics’, is employed to rapidly process the data.

In this part of the workshop we present the Lipidico injector and demonstrate how it works as part of the Australian Synchrotron SMX instrument. Expected outcome is shared knowledge, technical and practical know-how to perform a serial crystallography experiment at the Australian Synchrotron.

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Biography and Abstract

Andrew Peele

Professor Peele was appointed Director of ANSTO's Australian Synchrotron in November 2013. He is also an adjunct Professor of Physics at La Trobe with previous roles at NASA's Goddard Space Flight Centre and as a practicing solicitor. Andrew's research improves the versatility and quality of x-ray imaging, including over 100 publications including new methods in phase imaging and coherent diffractive imaging with applications such as tomographic imaging of cells and materials. He is a Fellow of the Australian Academy of Technology and Engineering (ATSE), and immediate past President of the Australian Institute of Physics and of the Asia Oceania Forum for Synchrotron Radiation Research. He also serves as a board member for the Australian Institute of Nuclear Science and Engineering and the Australian Institute of Mathematical Sciences and on advisory boards, including international synchrotron facilities, the La Trobe Institute for Molecular Sciences, and the ARC Centre of Excellence for Flexible Low-Energy Electronic Technologies.

Title: Crystallography at the Australian Synchrotron, ANSTO: status and plans

Abstract: The Australian Synchrotron, ANSTO has had great success in protein and chemical crystallography since its establishment. By staying current with technology and scientific developments the crystallography beamlines have managed a high rate of productivity even while hosted on a modest 3rd generation source. With a new crystallography beamline approved in principle, the facility is now entering an active phase of planning. Some developments in the field currently include, cryo-EM, XFEL crystallography and associated technology such as serial crystallography. By optimising the user offering with respect to competing and complementary technology a new beamline, as part of a suite of three beamlines, can continue to produce world-leading impacts at the Australian Synchrotron. In this context, some of the current thinking about the development of crystallography will be presented.

Henry Chapman

Henry Chapman is a director of the Center for Free-Electron Laser Science at the Deutsches Elektronen-Synchrotron and the University of Hamburg in Germany. He carried out his PhD in X-ray optics at The University of Melbourne, Australia, work for which he was awarded the Bragg Gold Medal from the Australian Institute of Physics. Henry develops methods in coherent X-ray imaging and in exploiting the short pulse durations and extreme intensities of free-electron lasers to obtain room-temperature macromolecular structures. He

is currently developing serial femtosecond crystallography using FEL and synchrotron radiation and extending it to the smallest possible crystals: that is, single molecules.

Title: Serial crystallography and diffraction with X-ray FELs

Abstract: Using X-ray free-electron laser pulses it is possible to outrun the effects of radiation damage, allowing room temperature measurements of macromolecular crystals at high resolution with a dose thousands of times higher than usually tolerable. Since an X-ray FEL pulse ultimately destroys the sample, measurements are carried out in a serial fashion, one sample at a time. This has led to the paradigm of serial crystallography, requiring rapid sample delivery, high frame-rate detectors and software to aggregate data into what is essentially a three-dimensional powder diffraction pattern. High-resolution room-temperature protein structures have been determined from crystals less than 0.01 micron^3 in volume, and high-resolution diffraction can be recorded from 2D macromolecular crystals or single fibrils. The method is especially useful for time-resolved crystallography, radiation-sensitive samples, small crystals, and studies of the dependence of structure on physical conditions and environments. The opportunities for this method have not been fully explored, and all aspects of the method are still under active development. I will outline some of these opportunities and developments.

Alexandra Ross

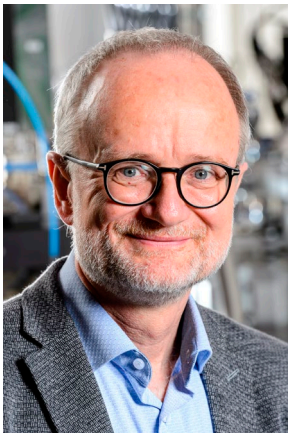
Dr. Alexandra Ros is Associate Professor in the School of Molecular Sciences and faculty member of the Center for Applied Structural Discovery (CASD) at the Biodesign Institute at Arizona State University. She received her Diploma in Chemistry from the Ruprecht-Karls University in Heidelberg, Germany, and her PhD from the Swiss Federal Institute of Technology, Lausanne, Switzerland. Since her PhD, Dr. Ros has been interested in microfluidic platforms and their analytical applications. Dr. Ros joined the Biophysics and Nanoscience Group at Bielefeld University, Germany, in 2000 where she followed her interests in microfluidics and biophysics during her post doctoral training. From 2001-2007, she served as principle investigator at Bielefeld University, Germany, on several projects investigating migration mechanisms and single cell analysis in the microfluidic format. In 2007, she finished her Habilitation and received the *Venia Legendi* in Experimental Physics from Bielefeld University. Dr. Ros joined Arizona State University in 2008 as Assistant Professor where she was promoted to Associate Professor in 2014. In the same year she became faculty member of the Center for Applied Structural Discovery (CASD) at the Biodesign Institute. In 2015-16, Dr. Ros was appointed visiting scientist at the Georg-August University Göttingen, Germany. She received a NSF Career Award in 2012 as well as a Fellowship for Experienced Researchers from the Alexander-von-Humboldt Foundation, Germany, in 2015 as well as the 2018 FACSS innovation award for her work on droplet microfluidics related to crystallography. Dr. Ros' current research interests include migration mechanisms in the micro- and nanoenvironment, single cell analysis, surface design and in particular the development microfluidic tools for crystallography. For the latter, her interests cover a wide range of applications including time-resolved crystallography, efficient and low sample consumption injection techniques, microfluidic approaches for crystallization as well as novel 3D printing approaches to facilitate crystallography applications.

Title: Microfluidic Tools for Serial Crystallography

Abstract: Serial Femtosecond Crystallography (SFX) with X-ray Free Electron Lasers (XFELs) is an emerging technique for protein crystallography. It has allowed exciting new insight into the structure of large

membrane protein complexes and enables time-resolved crystallography studies on light activated reactions in proteins. However, SFX with XFELs still faces serious limitations due to sample consumption and necessary injection techniques to allow mix and inject experiments for the study of protein substrate or ligand reaction dynamics. Microfluidic devices allow to address these limitations. First, droplet generation of the aqueous suspension containing the crystals of interest can be readily generated with microfluidic approaches. The synchronization of the generated droplets with the pulsed XFELs requires millisecond control of droplet release, which can be achieved with electrical triggering. Second, mix and inject experiments are facilitated through microfluidic mixers with hydrodynamic mixing strategies. This approach allows millisecond mixing times and >10 ms reaction time points. The versatility of designing such microfluidic devices is demonstrated through cutting-edge 3D printing technology, which also allows the integration of injection nozzles, such as the popular gas dynamic virtual nozzle. Some recent and future applications of these integrated devices for SFX with XFELs will also be discussed.

Robert Feidenhans'l



Robert Feidenhans´l received his Master's degree in Physics in 1983 and his PhD in 1986 both from the University of Aarhus. He worked as a staff scientist in the Physics Department at Risø National Laboratory from 1986-2001, where he became Head of the Materials Department also at Risø. In 2005 he became professor at the Niels Bohr Institute at University of Copenhagen, where he was vice institute leader 2007-2012 and Head of the Institute 2012-2017. January 2017 he became Managing Director of the European XFEL in Hamburg. Robert Feidenhans'l has been working in the field of X-ray Synchrotron Radiation and Free Electron Laser nearly all his career and has also been Chairman of Council at the European X-ray Radiation Facility in Grenoble and also at European XFEL. He is co-author of about 190 publications.

Title: European XFEL: First Results After 1.5 Years of Operation

Abstract: Hard X-ray Free Electron (XFEL) laser provide extremely and intense and ultra-short X-ray pulses that are ideal to investigate structural and dynamics of matter at very short time scales. X-ray free electron lasers have been in operation for 10 years now and have had wide range of areas of applications, including in structural biology.
European XFEL is the most recent large scale research infra structure in Europe taken into user operation in September 2017. It is a hard X-ray free electron laser and provides a very powerful X-ray beam for research. The European XFEL is an intergovernmental organization with 12 member states and is a facility that serves the European user community by providing the possibility for performing new classes of experiments to investigate the structure and dynamics of matter on the atomic length and time scales. The facility encompasses a 3.5 km long tunnel from DESY in Hamburg/Bahrenfeld to Schenefeld in Schleswig-Holstein where the experimental hall is placed. The tunnel encloses a 2 km long superconducting accelerator operated by DESY and undulator radiation sources. The first two experimental stations have been in operation doing user experiments for about one and a half years, one of them the SPB/SFX instruments for structural biology. In total six instrumental stations will be in user operation in the first half of 2019. In the talk the basic principles of the European X-FEL will be discussed and results of some of the first experiments will be shown.

Harry Quiney

Harry Quiney is Professor of Theoretical Condensed Matter Physics at The University of Melbourne. His background is in relativistic quantum electrodynamics, atomic and molecular physics and the theory of coherent diffraction imaging. The research of his group within the ARC Centre of Excellence for Advanced Molecular Imaging develops models of electrodynamical processes in materials exposed to intense femtosecond XFEL pulses.

Title: Plasma dynamics in XFEL pump-probe experiments

Abstract: In recent pump-probe XFEL imaging experiments on lysozyme crystals [1] it was found that bridging sulphur atoms exhibit anomalous dynamical behaviour. Over 100 fs, the structure of the molecule remained essentially intact, except for the migration of the sulphur atoms over a distance of 1-2 Angstrom. We have developed a plasma model to capture the evolution of electronic structure in the crystal over this timescale that is coupled with atomic physics models of photoionization, Auger decay, fluorescence and electron recombination. Qualitative agreement with the observed experimental behaviour is obtained in the case of the motion of the sulphur atoms. Further development of the model will be required to capture other observed effects, including the electronic polarization of carbonyl bonds, which involve molecular electronic structure effects.
[1] Ilme Schlicting, private communication.

Peter Berntsen

Dr Peter Berntsen completed his PhD in Material Physics at Chalmers University, Sweden, where he studied motions in biomembranes and rheology of human airway smooth muscle cells. In 2012 he joined Prof. Richard Neutze's group at University of Gothenburg, Sweden who uses X-ray methods to probe protein structures and their dynamics, and in 2015 he took up a research fellow position in at La Trobe University as part of the ARC Centre of Excellence in Advanced Molecular Imaging.
Dr Berntsen's research interests are focused on understanding the structural dynamics in biological molecules and in particular designing experiments in which a reaction is initiated and observed over time. To this end he is developing tools and sample delivery techniques for synchrotron and X-ray Free-electron Lasers (XFELs) to perform experiments that can lead to quantitative insight into biological processes.

Title: Serial Crystallography at the Australian Synchrotron

Abstract: A serial millisecond crystallography (SMX) facility has recently been implemented at the Macromolecular crystallography beamline, MX2 at the Australian Synchrotron. The setup utilizes a combination of an EIGER X 16M detector system and an in-house developed high-viscocity injector, `Lipidico'. Lipidico uses a syringe needle to extrude the microcrystal-containing viscous media and is compatible with commercially available syringes. The combination of sample delivery via protein crystals suspended in a viscous mixture and a millisecond frame rate detector, enables high-throughput serial crystallography at the Australian Synchrotron. A hit-finding algorithm, based on the principles of `robust-statistics', is employed to rapidly process the data.
Here we present details of the experimental setup, sample injector, and data analysis pipeline that are designed and developed as part of the Australian Synchrotron SMX instrument.