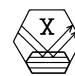


S4-L5

ADVANCEMENTS IN AUTOMATED IMAGING

Jochen Müller-Dieckmann

Formulatrix, 10 DeAngelo Drive, Bedford, USA

The unambiguous and reliable identification of biological crystals remains a major obstacle in crystallography particularly in the critical stage of initial screening experiments. Automated imaging at high-resolution in the VIS range is often insufficient to identify all conditions that have or may result in crystals. The use of UV light and fluorescence imaging adds an additional tool in that process. One possibility to overcome the shortcomings of intrinsic fluorescence, which critically depends of the amount of naturally occurring Trp, is Trace Label Fluorescence (TLF). A new protocol allows easy and rapid labelling of 0.1% of samples with a variety of fluorescent dyes immediately prior to crystallization. In addition to a significantly enhanced signal to noise ratio over intrinsic fluorescence, multiple labelling can be used to verify the presence of multi-subunit complexes in the crystal. We introduce a new device which enables users to quickly change between the required excitation wavelengths for their preferred dye or combination of dyes.

Second Order Non-linear Imaging of Chiral Crystals (SONICC) provides unprecedented resolution and contrast in the identification of chiral crystals entirely independent of any background material like soluble or precipitated sample or plastic ware. Microcrystalline material which is often the only lead in initial screening experiments can positively be identified. The method relies on the use of a femto-second laser at 1064nm wavelength and the rapid scanning of crystallization droplets to prevent sample damage. We have integrated that technology with our Rock Imager line of imagers to further enhance the repertoire of tools for the unambiguous identification of crystals in crystallography.

Both methods and their technical realizations will be introduced together with examples from the field to demonstrate their range of application and benefits.

Session V - Serial Crystallography

Monday, July 4 - morning

S5-L1

CHANGING CONCEPTS: CRYSTALLIZATION FOR SERIAL AND TIME-RESOLVED CRYSTALLOGRAPHY

Dominik Oberthuer

DESY, CFEL, Notkestrasse 85, Hamburg, 22607, Germany

Serial crystallography methods at both X-ray free-electron lasers (Chapman, 2011) and third generation synchrotron sources (Gati, 2013; Stellato, 2014) are now well established, making it possible to determine the structures of proteins – from small to very large – that only form very small crystals or that are extremely radiation sensitive. Serial crystallography methods are also very well suited for time-resolved experiments (Tenboer, 2014; Barends, 2015), making it possible to reveal the dynamic nature of biological macromolecules and their interactions at near-atomic spatial resolution and on ultrafast timescales. In case of such experiments one deliberately strives to grow micron- or sub-micron sized crystals of proteins that would otherwise form larger crystals as well. Small crystals not only allow for uniform laser excitation of all unit-cells in the X-ray beam, but for future mix-and-diffuse studies of reactions that cannot be photo-induced, since the small dimensions allow for fast enough diffusion of substrate into the crystals, such that enzymatic reactions can be induced homogenously at a defined time delay to the X-ray probe. Size homogeneity of the crystals and, since the amount of sample is limited, a high yield of crystals and optimized sample delivery methods are very important in this regard. Here promising new crystallization methods will be shown, as well as a pipeline for (nano-) crystal characterization prior to the crystallographic experiment and an overview on strategies to reduce sample consumption. Yet another paradigm shift that will be addressed is related not to the size, but to the internal quality of protein crystals: In the past it was attempted to grow as highly ordered protein crystals as possible, however recently it could be shown that certain disorder in protein crystals can be exploited to directly obtain the molecular transform from crystallographic experiments (Ayyer, 2016).

Ayyer, K. et al., (2016) Macromolecular diffractive imaging using imperfect crystals. Nature 530, 202-6.

Barends, T.R. et al., (2015) Direct observation of ultrafast collective motions in CO myoglobin upon ligand dissociation. Science 350, 445-50.

Chapman, H. N. et al., (2011) Femtosecond X-ray protein nanocrystallography. Nature 470, 73-77.

Gati, C. et al., (2014) Serial crystallography onin vivogrown microcrystals using synchrotron radiation. IUCrJ 1.

Stellato, F. et al., (2014) Room-temperature macromolecular serial crystallography using synchrotron radiation. IUCrJ 1, 204-212.

Tenboer, J. et al., (2014) Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein. Science 346, 1242-1246.

S5-L2

MICRO-CRYSTALLISATION OF PHOTOSYNTHETIC REACTION CENTER FOR TIME-RESOLVED SERIAL FEMTOSECOND CRYSTALLOGRAPHY AT AN X-RAY FREE ELECTRON LASER

Robert Dods

Gothenburg University, Chemistry and Molecular Biology, Gothenburg, Sweden

Advances at X-ray free electron lasers (XFELs) are broadening the scope of this technology and allowing experiments with more varied and difficult protein targets. One remaining bottleneck however is the production of micro-crystals suitable for delivery to the XFEL beam. Through a series of experiments at the Linac Coherent Light Source, California, we have optimised two different methods for micro-crystal production of the photosynthetic reaction center from *Blastochloris viridis*. One, in which we crushed macro-crystals mechanically through vortexing, and another in which we grew homogeneous samples of micro-crystals using a micro-seeding method. These methods have led to an increased crystal hit-rate and thus decreased sample usage compared to previous experiments on the same protein. Our micro-crystals diffracted to 2.85 Å, and were suitable for a time-resolved experiment, in which we visualised the protein at two time-points of the photosynthetic reaction. The micro-crystallisation techniques described will be valuable additions to the small but growing library of techniques available for structural biologists performing serial crystallography experiments at an XFEL

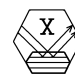
S5-L3

ADVANTAGES OF SERIAL FEMTOSECOND CRYSTALLOGRAPHY FOR RNA STRUCTURE DETERMINATION

J.R. Stagno[1], Y. R. Bhandari[1], C.E. Conrad[2,3], Y. Liu[1], M. Swain[1], Lixin Fan[4], G. Nelson[5], C. Li[5], D.R. Wendel[1], T.A. White[6], A. Barty[6], R.A. Tuckey[1], P. Yu[1], U. Weierstall[5], N.A. Zatsepin[5], T.D. Grant[7], C.D. Schwieters[8], J. Zhang[9], A. Ferré-D'Amaré[10], P. Fromme[2], D.E. Draper[11], K. Tan[12], X. Zuo[13], X. Ji[14], J.C.H. Spence[5] & Y.-X. Wang[1]

[1]Structural Biophysics Laboratory, National Cancer Institute, Frederick, MD 21702; [2]Department of Biochemistry, Arizona State University, Tempe, AZ 85287, USA; [3]Center for Applied Structural Discovery, The Biodesign Institute, Arizona State University, Tempe, AZ, 85287, USA; [4]The Small Angle X-ray Scattering Core Facility, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA; [5]Department of Physics, Arizona State University, Tempe, AZ 85287, USA; [6]Center for Free-Electron Laser Science, Deutsches Elektronen-Synchrotron DESY, Notkestraße 85, 22607 Hamburg, Germany; [7]Hauptmann-Woodward Medical Research Institute, Buffalo, NY 14203, USA; [8]Center for Information Technology, National Institutes of Health, Bethesda, MD 20892-5624, USA. [9]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, [10]Laboratory of RNA Biophysics and Cellular Physiology, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892, USA. [11]Department of Chemistry, Johns Hopkins University, Baltimore, Maryland 21218, USA. [12]Structural Biology Center, Biosciences, [13]X-ray Science Division, Advanced Photon Source, Argonne National Laboratory, Argonne, IL 60439, USA. [14]Macromolecular Crystallography Laboratory, National Cancer Institute, Frederick, MD 21702, USA.

Discovery of the important and diverse biological roles of RNA molecules is ever increasing. Similar to proteins, knowledge of the three-dimensional structures of RNAs is critical to understanding their functions. However, structure elucidation of RNAs using conventional methods has been extremely hampered by technical challenges, and is reflected in the overwhelmingly few RNA structures in the protein data bank. In addition to molecular size limitations



faced by nuclear magnetic resonance (NMR), the intrinsically similar chemical signatures of nucleotides result in severe peak overlap in NMR spectra. RNAs are often difficult to crystallize, and when available, RNA crystals often exhibit high mosaicity, high solvent content, and high susceptibility to radiation damage. In addition, RNAs tend to be very dynamic, and low-temperature data collection on a single crystal may not provide the most accurate depiction of its structure. Clearly, there is an acute need for advanced methods for RNA structure determination. Serial femtosecond crystallography (SFX) using an X-ray free electron laser (XFEL) has the potential to revolutionize RNA crystallography by overcoming many of these technical challenges. Its advantages include the use of nano/micro-sized crystals, room-temperature data collection, the ability to outrun radiation damage, and a high-throughput oversampling of crystal data. We have used SFX to determine the structure of the adenine riboswitch RNA apatamer domain in the ligand-free state. For the first time, these results provide a structural basis for the ligand-induced conformational switch required for the regulation of gene expression.

This material is based upon work supported by funds from the NIH Intramural Research Program.

S5-L4

GRAPHENE-BASED MICROFLUIDICS FOR SERIAL CRYSTALLOGRAPHY

Shuo Sui,[1] Yuxi Wang,[1] Derek MacPherson,[2] Vukica Srajer,[3] Robert Henning,[3] Jeanne Hardy,[2] Christos Dimitrakopoulos,[1] Sarah L. Perry[1]

[1] *Department of Chemical Engineering, University of Massachusetts Amherst, Amherst, MA 01003, USA*
[2]*Department of Chemistry, University of Massachusetts Amherst, Amherst, MA 01003, USA*
[3]*Center for Advanced Radiation Sources, University of Chicago, Argonne, IL 60439, USA*
perrys@engin.umass.edu

Coupling microfluidic technology with advanced protein crystallography techniques for *in situ* analysis is an area of research where significant advances can be made. Microfluidic platforms have the benefit of not only enabling experiments at small volumes, but also of creating an environment free of inertial or convective effects while providing exquisite control over local conditions and gradients. A significant problem in microfluidic-based crystallography is the background scattering resulting from the interaction of X-rays with the device materials, which may reduce the signal to noise ratio obtained from small or weakly diffracting crystals. These challenges can be overcome by decreasing the overall thickness of the microfluidic device. Graphene is an atomically-thin layer that can act as an X-ray compatible window material with negligible contributions to background scattering. Additionally, the remarkable mechanical strength and gas impermeability of graphene further enhance its utility for integration into microfluidic devices for protein crystallography. To facilitate handling and incorporation of atomically-thin graphene layers into a microfluidic chip, we utilize graphene attached to a ~200 nm PMMA supportive film using standard CVD graphene transfer techniques. We have evaluated the ability of various graphene-PMMA films to protect against sample dehydration over time, along with various hydrophilic surface treatments to facilitate surface wetting in microfluidic channels. The potential for these ultra-thin devices to enable data collection from microcrystals was evaluated using serial Laue crystallography of caspase-7, a member of the cysteine aspartate family of proteases that regulate apoptosis, or programmed cell death. This work demonstrates the utility of ultra-thin graphene-PMMA films as a sub-micrometre level barrier against dehydration in microfluidic devices while enabling the collection of high quality X-ray diffraction data on biomedically important proteins. Our ultimate goal is to utilize graphene-based microfluidic technologies and serial crystallography to enable the use of chemical triggering for the time-resolved structural analysis of additional biologically and biomedically-relevant protein targets.