Session VI - Crystal to Beam

Monday, July 4 - afternoon

S6-L1

A LOW BACKGROUND SAMPLE HOLDER FOR FIXED TARGET SERIAL CRYSTALLOGRAPHY EXPERIMENTS

A. Meents[1], P. Roedig[1], I. Vartiainen[2], R. Duman[3], S. Panneerselvam[1], N. Stuebe[1], O. Lorbeer[1], M. Warmer[1], G. Sutton[4], D. I. Stuart[3,4], E. Weckert[1], C. David[2], and A. Wagner[3]

[1]Deutsches Elektronen Synchrotron DESY, Photon Science, Notkestraße 86, 22607 Hamburg
[2]Paul Scherrer Institut, Villigen PSI, 5232, Switzerland
[3]Diamond Light Source Ltd., Diamond House, Harwell Science & Innovation Campus, Didcot, Oxfordshire, OX11 0DE, United Kingdom
[4]Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, United Kingdom
alke.meents@cfel.de

Serial crystallography has become a great success story and an increasing number of structures have been solved using this novel method [1]. In contrast to conventional crystallography, where a structure determination is ideally carried out by data collection from one large crystal in serial crystallography diffraction images from several thousands of small crystals are collected in a serial fashion. The individual datasets from all these crystals are then merged into one large and ideally complete data set from which the structure can be determined. Originally developed for X-ray Free Electron Lasers, serial crystallography has recently been applied at synchrotron sources.

Efficient sample delivery for serial crystallography remains challenging. Currently most of the experiments are conducted with liquid-jet systems with diameters down to 1 um. Drawbacks of all liquid jet delivery systems are high sample consumption and moderate hit rates of typically a few per cent. The use of high viscosity jets such as LCP or agarose tremendously reduces sample consumption but is paid with a large background scattering signal making this method impracticable for micron-sized crystals.

Another sample delivery technique for serial crystallography is the use fixed targets [2]. Here the crystals are loaded onto a solid support, which is then raster-scanned through the X-ray beam. We have developed a sample holder from single crystalline silicon and of about 2.5 x 4.5 mm in size, which is equipped with a periodic array of micro-pores with a diameter between 4 and 30 m [3]. For loading of the chips the micro-crystal-suspension is pipetted on the upper side of the chip. Mother-liquor is then soaked-off through the micro-pores side by using a piece of filter paper from the lower side of the chip. All micro-crystals larger then the pore diameter are retained by the chip and arrange themselves to periodic pore pattern. Drying out of the crystals is prevented by continuously exposing them to a stream of humidified air or helium during loading and data collection. For data collection at cryogenic temperatures the chips can be alternatively cryo-frozen by plunging them into liquid nitrogen or ethane after loading. Due to efficient removal of the mother liquor, the use of single crystalline silicon, and the absence of any cover foil, the chip allows for X-ray data collection with very low background. The chip has already been successfully used for several structure determinations at synchrotrons and also at the X-ray Free Electron Laser LCLS in Stanford.

1. H.N. Chapman *et al*. Femtosecond X-ray protein nanocrystallography. *Nature* **470**, (2011), 73–77.
2. A. Zarrine-Afsar, A. *et al*. Crystallography on a chip. *Acta Cryst.* D**68**, (2012), 321-323.
3. P. Roedig et al., A micro-patterned silicon chip as sample holder for macromolecular crystallography experiments with minimal background scattering, *Sci. Rep.* 5, (2015), 10451.

S6-L2

MICROCRYSTAL SAMPLE DELIVERY FOR SERIAL CRYSTALLOGRAPHY IN A HIGH VISCOSITY MEDIUM

Uwe Weierstall

Department of Physics, Arizona State University, Tempe 85287, USA

Serial crystallography at XFEL's has shown great promise in recent years for solving crystal structures from proteins which grow only micron sized crystals. G-protein coupled receptors are an important group of membrane proteins which are often crystallized in Lipidic Cubic Phase (LCP). This material has very high viscosity, and a device has been developed, which allows the generation of a microscopic stream of LCP with adjustable speed for sample delivery to the X-ray beam [1]. Some important GPCR structures could be solved with this device at the LCLS [2]. In addition, new media with similar viscosity to LCP have been developed which enable delivery of soluble or membrane proteins into the X-ray beam with low sample consumption [3]. The high viscosity injection method has also been shown to facilitate serial diffraction experiments with microcrystals at synchrotron microfocus beamlines. This talk will highlight these developments and discuss the possibilities.

1. Weierstall, U., James, D., Wang, C., White, T. A., Wang, D., Liu, W., et al. (2014). Lipidic cubic phase injector facilitates membrane protein serial femtosecond crystallography. *Nature Communications*, *5*. http://doi.org/10.1038/ncomms4309
2. Kang, Y., Zhou, X. E., Gao, X., He, Y., Liu, W., Ishchenko, A., et al. (2015). Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser. *Nature*, *523*(7562), 561–567. http://doi.org/10.1038/nature14656.
3 Conrad, C. E., Basu, S., James, D., Wang, D., Schaffer, A., Zatsepin, N. A., et al. (2015). A novel inert crystal delivery medium for serial femtosecond crystallography. *IUCrJ*, *2*(4), 421–430.

S6-L3

DISTINGUISHING PROTEIN NANOCRYSTALS FROM AMORPHOUS PRECIPITATE BY DEPOLARIZED DYNAMIC LIGHT SCATTERING

R. Schubert[1,2], A. Meyer[3], K. Dierks[3], S. Kapis[1], R. Reimer[4], H. Einspahr[5], M. Perbandt[1,2], Christian Betzel[1,2]

[1]University of Hamburg, Laboratory for Structural Biology of Infection and Inflammation, c/o DESY, Build. 22a, Notkestrasse 85, 22607 Hamburg, Germany
[2]Center of Ultrafast Imaging, c/o DESY, Build. 99, Luruper Chaussee 149, 22761 Hamburg, Germany
[3]XtalConcepts, Marlowring 19, 22525 Hamburg, Germany
[4]Heinrich-Pette-Institute, Leibniz-Institut für Experimentelle Virologie, Martinistraße 52, Hamburg, 20251, Germany
[5]PO Box 6483, Lawrenceville, NJ, 08648-0483, USA
Christian.Betzel@uni-hamburg.de

Growth and preparation of high quality micro-sized protein crystals, optimal for data collection experiments at modern micro-focus synchrotron (SR) beamlines and growth of nanocrystals required for data collection at Free-Electron-Laser (FEL) radiation sources is a new and challenging task. Latest methods will be presented to precisely monitor crystal growth and to optimize the preparation of crystalline particles, too small to be observed by light microscopy. The identification of the presence of a spatial repetitive orientation of macromolecules (crystal nuclei) in the early stages of the crystallization process is essential to detect nanocrystals. The optical properties of a crystal lattice offer the potential to detect the transition from disordered to higher ordered particles. A unique experimental setup was designed and constructed to detect nanocrystal formation by analyzing depolarized scattered laser light. The ability of a lattice to depolarize laser light depends on the different refractive indices along different crystal axes. The results obtained so far demonstrate that the successful detection of nano-sized protein crystals at early stages of crystal growth is possible, by analyzing the signal intensity of the depolarized component of the scattered light. The method and approach allows an effective differentiation between protein-dense liquid cluster formation and ordered nanocrystals [1]. The data and results obtained so far were verified by complementary methods like X-ray powder diffraction, second harmonic generation, ultraviolet two-photon excited fluorescence and scanning electron microscopy.

Further, this particular advanced laser light scattering technique can be combined with a state of the art protein crystallization robotic setup (Xtal-Controller [2]), allowing the controlled nanoliter increments addition of protein, precipitant and additive solution towards a crystallization so-

lution sitting on a microbalance. By this combination, crystallization phenomena can be characterized in detail and methods can be optimized for the efficient production of nanocrystals. Details and examples will be presented.

The investigation were supported by the excellence cluster "The Hamburg Centre for Ultrafast Imaging - Structure, Dynamics and Control of Matter at the Atomic Scale" of the Deutsche Forschungsgemeinschaft (DFG) and by the Röntgen-Angström-Cluster (project 05K12GU3) funded by the German Federal Ministry of Education and Research (BMBF).

1. Schubert *et al.,* Journal of Applied Crystallography, Issue 48, 1476-1484, (2015)
2. Meyer *et al.* Acta Crystallographica Section F68, 994-998, (2012).

S6-L4

ACOUSTIC DROPLET EJECTION: FROM CRYSTALLIZATION THROUGH TIME-RESOLVED SFX

Allen M. Orville

XFEL Hub at Diamond, Diamond Light Source, Diamond House, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE, United Kingdom

On demand acoustic droplet ejection is a general, touchless method that use focused sound waves to eject picoliter to nanoliter volumes from one place to another. A spot-focused piezoelectric transducer is driven by a waveform pulse with frequency and amplitude that ultimately impact droplet size and velocity, respectively. A wide variety of solutions can be manipulated, from crystallization reagents and chemical libraries to relatively viscous slurries of microcrystals. Droplets can be ejected from any well within a range of microplates, or launched from a custom, 3D printed and refillable micro-well. The droplet destination can be any well within a microplate, a known location such as a mounted crystal or any spot within MiTeGen micromesh, an optically and X-ray transparent transport belt, or directly into the XFEL interaction zone. Several examples of applications will be described, including on-demand acoustic injectors for SFX and spectroscopic data collection at the LCLS.

S6-L5

THE CHANGING ROLE OF IN-HOUSE CRYSTALLOGRAPHY

V. Smith, S. Freisz, M. Benning

Bruker AXS GmbH; Karlsruhe, Germany
vernon.smith@bruker.com

Continuing technical advances at synchrotron beamlines and the rise of XFELs is enabling structural biologists to tackle ever more challenging questions and has brought about major changes in the crystallisation-crystallography-structure-biology workflow.

This has led to a change in perception toward the usefulness of classical in-house crystallography and driven major technological advances required to provide in-house crystallographic facilities that are relevant to the modern experiment.

This talk will review how the development of techniques such as microcrystallography, serial crystallography and *in situ crystallography* at beamlines has been initiated development of new technologies for in house crystallography systems and discuss how the role of in-house crystallography has changed over recent years.