

MS1-03 Latest Methods to grow and prepare micro- and nano-Crystals for future Free-Electron-Laser and Synchrotron Radiation Sources. Christian Betzel^a, Arne Meyer^b, Karsten Dierks^b, Howard Einspahr^c, Rolf Hilgenfeld^b, Lars Redecke^b, Michael Duszynski^d, Henry Chapman^a ^aUniversity of Hamburg, Germany, ^bUniversity of Lübeck, Germany, ^cP. O. Box 6483, Lawrenceville, New Jersey 08648-0483 USA, ^dUniversity of Tübingen, Germany
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Growth and preparation of high quality micro-crystals optimal for data collection experiments at modern micro-beam insertion-device synchrotron (SR) beamlines and growth of nano-crystals required for data collection on an Free-Electron-Laser (FEL) beamline is a new challenging task. In the field of protein crystallization several fully automated instruments are available today and the search for crystallization conditions of macromolecules can easily be carried out. Nevertheless, to identify optimal growth conditions to obtain high quality X-ray suitable crystals still remains a bottleneck in most cases [1,2]. Considering the tremendous advantages of the new and upcoming high brilliant SR and FEL radiation sources, allowing to collect diffraction data from micro- or nano- crystals by conventional single crystal diffraction or via the new method of Serial Femtosecond Crystallography (SFX) [3] new crystallization and crystal scoring techniques need to be established.

To grow, score and prepare high quality nano- and micro-crystals we developed and tested new and advanced methods during the last years. In one approach we optimized the growth of nano sized crystals *in vivo*, in cells and in a second approach we developed an advanced hardware combination allowing the controlled optimization of a single drop vapour diffusion experiment for production of nano- and micro- crystals.

Protein crystallization in cells has been observed several times in nature and crystallization is known to occur also as a native process *in vivo*. Prominent examples include storage proteins in seeds, enzymes within peroxisomes and insulin within secretory granules. However, owing to their small size these crystals have so far not been used for X-ray crystallographic analysis. We recently prepared nano-sized *in vivo*-grown crystals of a Trypanosoma brucei enzyme and could show that the emerging free-electron laser based SFX method is suitable to record interpretable diffraction data and to solve the structure using XFEL data [4]. The unique combination of *in vivo* crystallization and SFX offers new possibilities to analyse proteins that do not form crystals suitable for conventional X-ray diffraction *in vitro* and will open new routes in structural biology [5].

The hardware allowing controlled vapour diffusion experiments is named “Xtal-Controller”, it permits charting a course across the phase diagram to produce nano- and micro- sized crystalline samples optimized for diffraction experiments. The optimization experiment is conducted with a single microliter-scale sitting drop device that is precisely temperature and humidity controlled and allowing a software-controlled evaporation to increase the concentration of the solution components in the sample drop. The sample drop is positioned on a microbalance and continuously observed optically and, importantly, by means of integrated dynamic light-scattering (DLS) [6]. The device allows determination of the phase at any position in the diagram because the presence or absence of nuclei is monitored *in situ* by DLS. Details and examples will be presented.

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MS1-04 Smart materials for protein crystallization. Naomi E. Chayen, *Biomolecular Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, London SW7 2AZ, UK*
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This presentation reports a unique means of producing protein crystals by designing “smart materials”, namely molecularly imprinted polymers (MIPs) as templates for crystallization. The idea was to find a material that would specifically attract protein molecules to come together and form a crystal. MIPs are polymers prepared in the presence of a molecule that is extracted afterwards, leaving complementary cavities behind. The cavities retain a memory of the molecule in the polymer and exhibit highly selective re-binding of the given molecule. Imprinting with protein molecules creates a fingerprint of the protein on the polymer, thereby enabling the MIP to serve as a tailor-made nucleant for crystal formation. MIPs are effective in both the screening and optimisation stages of crystallization [1]. MIP-containing screening trials (in a widely used commercial screen) yielded promising hits for proteins that had not produced useful crystals or indeed any crystals at all in their absence or in the presence of traditional nucleants. At the optimization stage, the presence of MIPs has led to faster formation of crystals in all cases where crystals would appear eventually and to major improvement in diffraction in some cases. The rationale and application of this new technique will be discussed.

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