Session IV - Automation in Crystallization

Monday, July 4 - morning

S4-L1

AUTOMATED CRYSTAL MOUNTING AND PROCESSING THROUGH LASER PHOTOABLATION. NEW OPPORTUNITIES FOR INTEGRATED MACROMOLECULAR CRYSTALLOGRAPHY PIPELINES

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The introduction of fast pixel array detectors is opening new opportunities and increasing the capacity of modern synchrotron facilities for macromolecular crystallography. However, the preparation of crystals for diffraction experiments still requires manual, elaborate manipulations that can result in sample loss. In collaboration with the EMBL instrumentation team we have developed a novel approach called CrystalDirect that enables fully automated crystal mounting and cryo-cooling [1-3]. This new technology is based on the use of a thin film as crystallization support from which crystals can be recovered by excising the film around the sample with a laser beam and attaching it to a data collection pin. By eliminating manual procedures, crystal mounting becomes a more reliable and controlled operation that does not depend on the skills of the scientist. Moreover, this approach offers an unprecedented level of control during sample processing opening a number of new possibilities. For example, a modification of the CrystalDirect protocol makes it possible to deliver small molecules and other chemicals to crystals by diffusion, providing an alternative to manual crystal soaking experiments. The CrystalDirect approach can contribute to closing the automation gap between crystallization and X-ray data collection thereby contributing to the advancement of challenging projects in structural biology that require the analysis of large numbers of crystals, like those targeting multi-protein complexes, membrane proteins or those involving large scale compound and fragment screening in the context of drug design campaigns. The experience from the use of this system at the High Throughput Crystallization Facility of the EMBL Grenoble outstation as well as the new opportunities enabled by the integration of crystallization and X-ray data collection into continuous, fully automated workflows will be discussed.

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2. J. A. Marquez, and F. Cipriani, *Methods Mol Biol,* 1091 (2014), 197-203.
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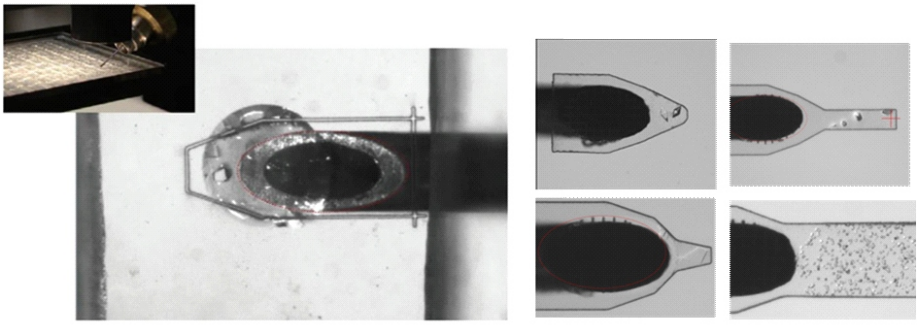

Figure 1. The left panel illustrates the principle of the CrystalDirect technology. The right panels show examples of crystals automatically mounted and cry-cooled.

S4-L2

ADVANCED IMAGING IN LAB-SCALE PROTEIN CRYSTALLIZATION

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Crystallization of difficult targets is a key bottleneck in structural biology and requires high-throughput and high-quality screening. Screen preparation has already reached a high level of automation, but crystal detection still largely relies on visual inspection and expert opinion. Advanced imaging modes, such as second-order-non-linear imaging of chiral crystals (SONICC), UV-two photon excited fluorescence (UV-TPEF) and trace-label fluorescence detection, provide tools for optimizing crystal detection and characterization. An overview on two and a half years of routine operation of an advanced imaging system employing all above mentioned imaging modes in a lab-scale environment is provided. Strategies for optimized crystal screening for challenging targets including nanocrystals, lipidic phase crystallization of membrane proteins and large protein assemblies, as well as for rapid optimization of routine protein-ligand co-crystallization are discussed.

S4-L3

TeXRank: TEXTURE IMAGE ANALYSIS AND MACHINE LEARNING FOR CRYSTALLIZING DIFFICULT PROTEINS

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While automation of both crystallization and drop imaging is now widespread, the flood of data remains largely unexploited. Algorithmic approaches have historically focused on per-drop scoring or else retrospective data mining; it remains unclear what impact the resulting computational tools have had on daily practice. We revisited drop analysis using modern texture methods, in particular the texton technique, with the ultimate goal of quantifying a protein's precipitation behaviour across the entire set of drops in a coarse screen, to serve as a fingerprint of its crystallizability that can be compared to the vast dataset of historical experiments at the SGC. The freely available program TeXRank comprises a suite of computational tools for day-to-day analysis of crystallization experiments, including: ranking of drops by likelihood of crystallinity; clear-drop analysis for discovery of better buffer solutions for the protein; and multi-drop analysis to provide a read-out of a protein's usefulness even in the absence of crystals along with pre-calculated optimisation screens. These tools will be described and results discussed, along with questions of uptake amongst users pre-conditioned to expect very little from a given experiment, and further possibilities opened by these approaches.

S4-L4

MICROFLUIDIC PLATFORM FOR OPTIMISATION OF CRYSTALLISATION CONDITIONS

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Over the past few decades, the science of crystallisation has attracted vast interest in industrial and academic applications. In order to get suitable quality crystals, the crystallisation process is monitored and controlled through the screening of favourable crystallisation conditions and subsequent optimising of crystal growth by developing a specific kinetic path in the phase diagram. In recent years, crystallisation of proteins or pharmaceuticals has been improved thanks to high-throughput techniques, offering multiple operations such as mixing, analysis, separation. In one hand, high-throughput robots rely on automated liquid-handling techniques integrated with dispensers and fluidic circuits [1]. In return they generally require high budget. In the other hand, microfluidic techniques controlling and manipulating flows at even lower scales (micro, nano to picoliter) use miniaturised devices called lab on chip [2, 3]. However, these devices are not very accessible to non-specialists of microfluidics due to their complexity.

We develop a microfluidic platform accessible to non-specialists of microfluidics and applicable to soluble and membrane proteins in aqueous and organic solvents. It is based on the notion of modular microfluidics [4], with discrete components that can be easily assembled, replaced or removed in a plug-and-play mode. It includes modular sub-functions such as droplet generation and storage, on-line UV characterisation and observation (Fig.1). Thus, compared to traditional methods, it offers advantages with respect to system cost, planning, and maintenance.

We used this platform for the optimisation of crystallisation conditions of a protein. Therefore, the chemical composition, size and frequency of microdroplets were measured on-line by UV spectrophotometry. An initial condition was deduced from a rapid screening. Then we varied the concentrations of protein and crystallisation agent in hundreds droplets of identical sizes and we observed their influence on the crystallisation until we obtained 1 crystal per droplet. We expect this platform will be easily incorporated into any laboratory, such as chemical, pharmaceutical, biological.

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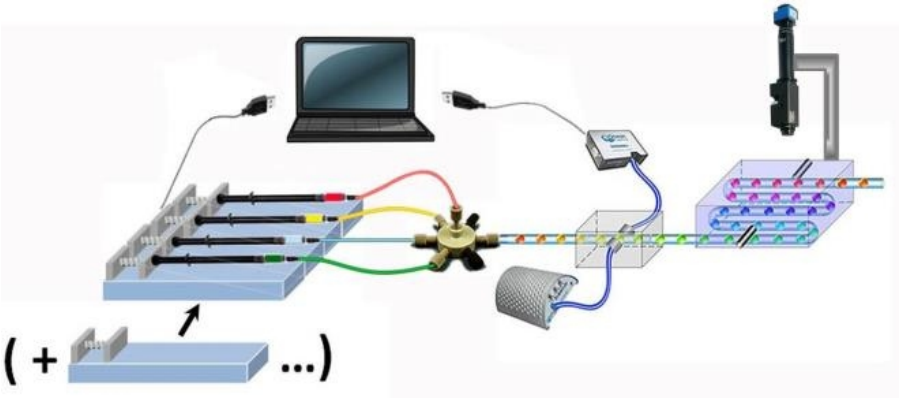

Figure 1. Scheme of the home-made microfluidic platform: (1) syringe pump, (2) 7-entry junction, (3) on-line UV module (4) tubing-holder for thermostatting and observation and XYZ-motorized camera.

S4-L5

ADVANCEMENTS IN AUTOMATED IMAGING

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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

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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.