The SLS crystallisation platform at beamline X06DA

A fully automated pipeline enabling *in situ* X-ray diffraction screening

Introduction

X-ray crystallography is the most widely used technique for macromolecular structure determination. However, it requires the production of suitable crystals, an iterative and time-consuming process which remains unpredictable. Hence, the rational of the Crystallisation Facility at the Swiss Light Source at the Paul Scherrer Institut was to streamline the steps between intial crystallisation hits and respective X-ray diffraction images, taking advantage of full integration into the synchrotron beamline X06DA. This unique and fully automated set-up allows initial crystals to be tested for their X-ray diffraction characteristics *in situ, i.e.* directly in the crystallisation container without post-crystallisation treatment, which could harm the crystal and hamper diffraction.

Structural biologists can easily distinguish desired macromolecular crystals from unwanted salt crystals and obtain rapid feedback on diffraction quality and important parameters such as limit of resolution, anisotropy, cell parameter and mosaicity.

In situ X-ray diffraction thus helps to better prioritise on subsequent crystal optimisation trials and can, in a few cases, even lead to a complete crystallographic dataset. The Crystallisation Facility at the SLS is therefore of particular interest for both academic and industrial users involved in structural biology activities.

List of services and equipment provided by the Crystallisation Platform

Macromolecular crystallisation

The SLS crystallisation platform is equipped with a Hamilton Star Plus liquid handling robot that covers the whole workflow of macromolecular crystallisation. Experiments are set up in SBS standard vapour diffusion containers with crystallisation drop sizes down to 100 nL. Users can choose from a range of 15 commercial crystallisation screens and 2 commercial additive screens, or dedicated optimisation screens can be requested based on initial

Figure 1: ***In situ*** **diffraction screening set-up at beamline X06DA at SLS PSI.**

crystallisation hits. SLS staff at the crystallization facility will support users in designing their optimisation screens. In order to support membrane protein crystallisation, future upgrades include a Mosquito nanolitre pipetting robot (TTP Labtech) designed for crystallisation in lipidic cubic phase (LCP).

Crystal imaging

The crystallisation experiments are stored and imaged in a Formulatrix Rockimager 1000 at 20° C, which is directly adjacent to the experimental hutch of beamline X06DA. The standard imaging schedule is after 1 hour, 24 hrs, 72 hrs, 7 days, 14 days, 4 weeks and 12 weeks. Observation of the cristallisation drops is performed using the web-based software RockmakerWeb, and *in situ* X-ray diffraction experiments of crystallisation hits can then be requested.

***In situ* X-ray diffraction screening at X06DA**

The macromolecular crystallography beamline X06DA at the SLS is equipped with a sample changing robot (Irelec CATS), which is endowed with a gripper designed to hold and position all SBS standard crystallisation containers (including microfluidic devices such as the Fluidigm diffraction-capable TOPAZ® chip and CrystalHarp™ counter-diffusion plates) in the X-ray beam (Figure 1). Crystallisation experiments that were set up and stored at the SLS facility can be transferred from the imaging system into the experimental hutch of beamline X06DA in a completely automated manner. Users not utilising the SLS protein crystallisation platform are also welcome to bring their own SBS standard crystallisation containers, to carry out *in situ* X-ray diffraction screening as part of their assigned beamtime or as a service from the crystallisation facilty staff. Due to the high photon flux (5e11 phs/sec) and focused X-ray beam (80×45 microns) at X06DA, crystals down to a few microns and cristalline precipitate can also be tested.

Examples of in situ diffraction screening

- Fast discrimination between salt (Figure 2 A, B) and macromolecular crystals (Figure 2 C, D, E, F, G, H)
- Assessment of diffraction quality from crystals, identification of the best crystallisation conditions or searching for new space groups (Figure 2 C, D)
- Identification of small and low diffracting crystals, including crystalline precipitate or nanosized crystals (Figure 2 G, H)

http://sls.web.psi.ch/view.php/beamlines/px3

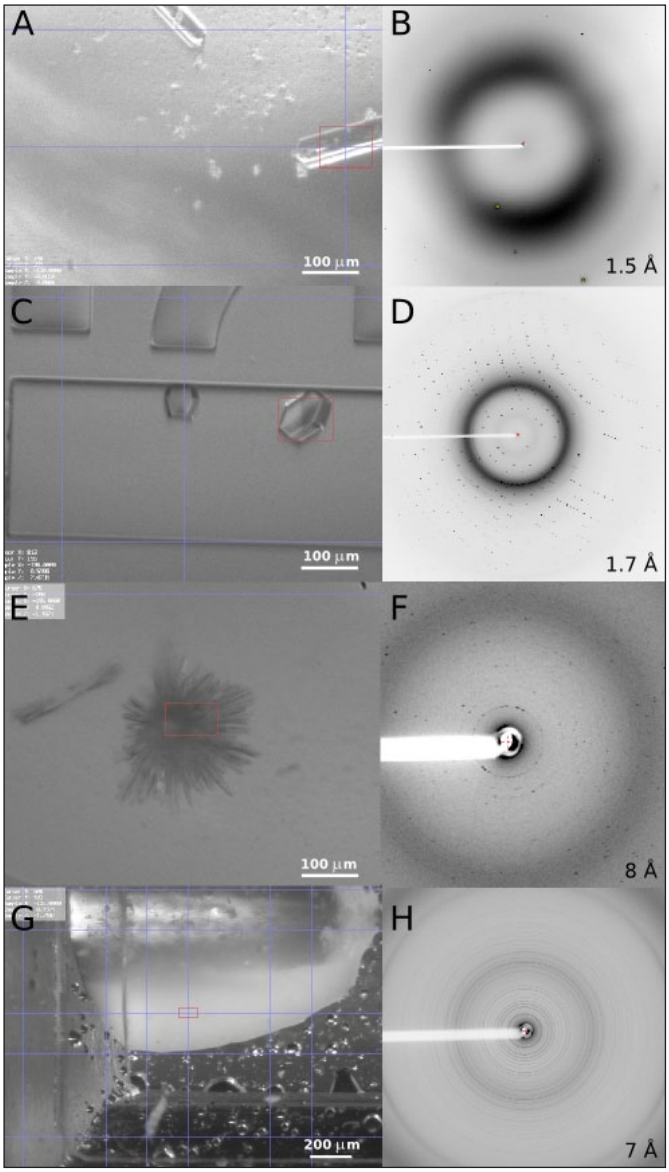

Figure 2: **Examples of *in situ* diffraction screening experiments.**

(A, B) Salt crystal and corresponding diffraction up to 1.5 Angs;

(C, D) Protein crystals and corresponding diffraction up to 1.7 Angs;

(E, F) Cluster of thin protein needle crystals and corresponding powder-like diffraction up to 8 Angs;

(G, H) Nanoprotein crystals (400 to 800 nm) in the crystallisation drop and corresponding powder-like diffraction up to 7 Angs.

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