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Serial synchrotron crystallography at EMBL PETRA III beamline P14.

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Serial synchrotron crystallography (SSX) combines single-shot X-ray images of randomly oriented crystals, recorded at a synchrotron source, into a single dataset. The method requires a high-brilliance synchrotron source with a beam size similar to the sample size, an appropriate sample delivery method, a detector with sufficient frame rate and a data processing pipeline. SSX can act as a pre-screening method for samples intended for XFEL experiments or a stand-alone experiment to determine the protein structure when crystal growth to larger size cannot be achieved, e.g. in vivo grown crystals. Proof of principle experiments [1] have shown the feasibility of the method.

Our SSX setup at the EMBL beamline P14 on the PETRA III storage ring at DESY (Hamburg, Germany) utilizes the sample delivery systems known from conventional crystallography - thus setup time is negligible and sample consumption is very low. SSX experiments can be done in situ in CrystalDirect™ plates, also in meso, possibly using the same plate the initial crystals grew in. Cryo-samples are mounted in loops or with the CrystalDirect™ Harvester. Generally, the sample size for a feasible experiment is a few microns. Data collection runs as series of helical line scans, typically a dataset is collected in a few minutes, depending on the size of the region of interest, crystal size and the detector used (Pilatus6M, Eiger4M, Eiger16M). Progression of the data collection is monitored throughout the experiment as on-the-fly calculated heat map, displaying the diffraction scores as estimated by program DOZOR [2].

The acquired diffraction images are sorted and bunched into sub datasets according to the DOZOR score using a script. The script generates XDS-processing files for each sub dataset and processing of all of these can be launched in parallel. Data is scaled using XSCALE. Data processing takes typically 30 minutes on good quality data. We have demonstrated the feasibility of the pipeline using 5-10 micron lysozyme and insulin crystals as test objects. Using an Eiger4M detector, a data set of 65120 images was collected at P14 in 3 minutes as an in situ experiment of 5 micron lysozyme crystals grown and presented to the beam in a CrystalDirect™plate. About 2000 sub dataset, each containing 5-10 diffraction images, were integrated and scaled to yield complete data to 1.7 Å resolution. The structure could be solved by molecular replacement and electron density maps were of good quality. Beamtime for SSX experiment can be applied for through the EMBL user program at https://smis.embl-hamburg.de.

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