Serial, in-situ-, time-resolved and large complex crystallography at P14/PETRAIII

Gleb P Bourenkov*, Isabel Bento, Johanna Hakanpää, Stefan Fiedler, Ivars Karpics, Marina Nikolova, Maxim Polikarpov, Guillaume Pompidor, and Thomas R. Schneider

EMBL Hamburg, Germany

gleb@embl-hamburg.de

The undulator beamline P14 is a part of the Integrated Facility for Structural Biology operated by the European Molecular Biology Laboratory at the PETRA III storage ring at DESY (Hamburg, Germany). The beamline is tunable across the energy range of 6-30 keV. The collimating/focusing optics at P14 comprise a white-beam compound-refractive lens (CRL) transfocator in 2:1 geometry, and a Kirkpatrick-Baez (KB) mirror system with 60:1(h)/1.5(v) demagnification. The beamline operates in two modes: collimated and micro-focusing, between which the user can toggle within 20 seconds. In collimated mode, the CRLs are used to create a top-hat beam, which can be shaped to any size between 20 and 300 μm at the sample position. The flux density is routinely adjusted to $5x10^{14}$ photon/s/mm^2 corresponding to a sample life-time of c.a. 100 seconds as required for high-quality single-crystal data collection with an EIGER 16M detector. The collimated top-hat beam allows homogeneous illumination of large crystals excluding hot-spot induced radiation damage. As demonstrated by the crystal structure determinations of the human 20S proteasome at 1.8 Å[1] and the core mediator complex at 3.4 Å resolution [2], such beam conditions are of particular interest for crystallography of large macromolecular complexes.

In micro-focusing mode, the use the KB-mirrors in combination with pre-focusing by the CRLs allows to increase the photon flux to up to 1.3 10^{13} ph/s into a 5-10 μm focal spot. The KB mirrors are currently being upgraded to reduce the focal spot size to 1-2 μm. High peak dose rates of up to 200 MGy/s enable successful applications of serial synchrotron crystallography [3]. In this method, hundreds- to thousands of microcrystals are rapidly rastered by several ten thousand rotation exposures. The method is integrated into a highly automated workflow with a high level of user control and interactivity. The beamline software supports the efficient handling of huge data flows and real-time data evaluation. Cryo-cooled crystal suspensions, *in-situ* crystals as grown in CrystalsDirect^{TM} plates and a number of customized setups[4] are used for sample delivery. Successful *in-situ* structure determinations have been performed on crystals grown in lipidic mesophase and in living cells.

Recently, we demonstrated that propagation-based phase contrast X-ray imaging can be used as an efficient tool for visualization of (micro-)crystals embedded in an opaque lipid phase at 100 K. As compared to standard diffraction-based X-ray centring, the method requires 100-fold lower dose while providing 10-fold higher resolution. This capability originates from the easy accessibility of the collimated mode at P14 and is now being integrated as a tool into the standard user interface, the MxCuBE.

In-situ- and room temperature serial methods are frequently used in laser-triggered time-resolved experiments on a *m*s-second time scale. A second P14 end-station for time-resolved pump-probed experiments is currently under construction.

References

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[3] Gati et al., (2014)*IUCrJ***1:**84-86.

[4] Schubert et al. (2016)*IUCrJ3:*393-401.