

Enabling high-energy, large-unit-cell, ultra-high-resolution X-ray crystallography on beamline P14@PETRAIII

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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). At high energies, a white beam compound refractive lens translocator in 2:1 geometry is used to deliver a top-hat beam that can be shaped to any size between 20 and 300 μm at the sample position. Variable-size top-hat beams are particularly important in structural studies of large macromolecular complexes [1] and ultra-high-resolution studies of enzymatic mechanisms under precise dose control [2].

In early 2021, the Dectris CdTe 16M EIGER2 detector was installed at the P14 beamline. A high-flux collimated mode at an energy of 26.687 keV was established as a new standard beamline setting, in order to boost data quality by reducing radiation damage [3]. To further improve data quality, Global Phasing Ltd's workflow has been deployed on P14 through its interface to MXCuBE2 ([4], § 4.4.7). Crystal symmetry and orientation are first determined, then used together with knowledge of the MD3-goniostat's reorientation capabilities to design a multi-orientation strategy aiming to achieve completeness (no cusps) and uniformity of redundancy, within a "dose budget" adapted to the target resolution. The workflow then directly drives the execution of that strategy via MXCuBE2.

The potential of the combined use of these technical advances is highlighted by two ultra-high-resolution datasets from large-unit cell systems: 1) A primitive orthorhombic system with 560 kDa per asymmetric unit (a.u.), that yielded a 0.98 Å resolution dataset (almost 100M reflections, 2.75M unique, at a dose of 2.5 MGy); 2) A C-centered orthorhombic system with 280 kDa per a.u., yielding a 0.96 Å resolution dataset (over 40M reflections, 1.375M unique, 2.5 MGy). Data collection times, including characterization and strategy design, were 15 minutes for both datasets.

We conclude that the combination of optimized beamline optics, an advanced data-collection workflow, and CdTe detectors with small pixels enables the collection of highly precise and accurate high-resolution data on large unit-cell systems at high energies, capable of supporting the most demanding applications of X-ray crystallography to Structural Biology, and enzyme mechanistic studies. The increasing availability of high-energy beamlines will allow for the standardization of the setup to the benefit of structural researchers.

[1] Singh et al. (2020) *Cell* 6, 10-13.

[2] Dai et al. (2019) *Nature* 573, 609-6

[3] Storm et al. (2021) *IUCrJ* 8(6)

[4] Oscarsson et al. (2019) *Journal of Synchrotron Radiation* 26, 393-405.