

Bridging the gap. Serial crystallography at 4th generation synchrotrons: current status and future goals

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Time-resolved serial crystallography (TR-SX) is a leading technique for capturing biological processes as molecular movies on extremely fast timescales—fulfilling a long-standing goal in structural biology. The method involves delivering microcrystals into a powerful, pulsed X-ray beam to collect individual diffraction patterns from each crystal. By compiling thousands of these patterns, researchers can reconstruct an electron density map. This technique, known as serial crystallography, allows for the observation of structural changes. When ultrashort X-ray pulses are used, SX can track time-dependent conformational changes, enabling scientists to visualize proteins in motion. TR-SX experiments are typically synchronized with a specific stimulus that initiates the biological activity under investigation. The advent of diffraction limited storage rings - the so-called 4th generation synchrotrons - have permitted the conception and built instruments that overcomes the limits of traditional microfocus beamlines. These new beamlines aim to exploit X-ray pulses down to microsecond time resolution, becoming an invaluable tool for room temperature and time-resolved studies that complements the capabilities of Free Electron Laser sources. ID29 at the European Synchrotron Radiation Facility is one of the first examples of this new generation of beamlines [1]. The unique combination of microsecond exposure times, advanced beam properties, and a flexible sample environment enables the collection of high-quality, complete data—even from exceptionally small amounts of crystalline material. This is applied in combination with external stimuli to activate or induce structural changes that could be observed in the microsecond time regime. This approach is particularly successful for the study of enzymatic reaction or ligand binding, thus prominently interesting for the whole structural biology community, while future developments will be crucial to strengthen the application of the methods to structural based drug design.

[1] Orleans, J. (2025). *Communications Chemistry*, **8**, 1. <https://doi.org/10.1038/s42004-024-01404-y>