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Advancing macromolecular structure determination with microsecond X-ray pulses at a 4th generation synchrotron

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Serial macromolecular crystallography (SMX) has become a powerful method for resolving the structures of biological macromolecules at room temperature (RT). Although microfocus beamlines at third-generation synchrotrons are instrumental, their data acquisition is typically limited to the millisecond scale due to constraints in photon flux and detector speed. The newly developed ID29 beamline at the European Synchrotron Radiation Facility (ESRF)—a flagship of the Extremely Brilliant Source (EBS) upgrade—was purpose-built to leverage the capabilities of this fourth-generation source. As the first beamline dedicated to room-temperature serial microsecond crystallography (RT-SμX) with true microsecond X-ray pulses, ID29 features a compact, flexible diffractometer that supports rapid sample exchange and accommodates multiple solid supports and three types of high-viscosity extruders (HVEs). Our study highlights the critical integration of pulsed beams, the fast JungFrau4M detector, and synchronized data acquisition systems for effective RT-SμX experiments. The unique beam properties of the new ESRF source enable microsecond time-resolved crystallography, yielding high-quality electron density maps from relatively few merged frames. These advances position RT-SμX at ID29 as a model for future applications at upcoming fourth-generation synchrotron facilities worldwide.

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