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Time-resolved macromolecular crystallography using the single pulse Laue technique

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Laue diffraction patterns from protein crystals with an exposure time of ca. 150 ps are now routinely acquired at the White Beam Station (ID9) of the European Synchrotron Radiation Facility (ESRF) by using the single bunch mode of the storage ring. The single pulse (SP) Laue technique opens new opportunities in the field of time-resolved macromolecular crystallography, since it allows one to follow in real time, at physiological temperature, tertiary structural modifications involved in protein dynamics. This work concentrates on two aspects.

First, the broad bandpass SP Laue camera developed on ID9 will be described: this camera is able to select single pulses of x-rays in synchronization with 7 ns laser pulses initiating a photo-reaction in the sample. The optics of ID9 have been designed to deliver maximum x-ray power at sample position. In the 15 mA single bunch mode of the machine, it is possible to use two insertion devices in series and up to 1.8×10^7 photons/0.1%bw can be delivered at 15 keV on a 0.2 x 0.2 mm^2 sample in a single 150 ps x-ray pulse.

Second, we will show that such a photon flux can give accurate structural information on the nanosecond timescale. We used cutinase, a 22 kDa lipolytic enzyme whose structure is known to 1.0 Å resolution. From a SP Laue data set with a total exposure time of 8.5 ns, the structure of native cutinase could be determined to 1.5 Å . In order to mimic a fast time-resolved experiment, we used as a starting model for refinement the structure of the mutant R196E, which crystallizes in a different space group. A satisfactory model of native cutinase was obtained, with R_{cryst} = 19.3% (R_{free} = 24.2%). Discrepancies between this model and the assumed "true" structure of cutinase (obtained from monochromatic data collected to 1.0 Å resolution, R_{cryst} = 9.5%) were minor. The wild-type Arg196 could be readily positioned in the electron density, and significant main and side-chain displacements due to packing constraints were successfully retrieved with the Laue data. The electron density maps showed good connectivity and were of sufficient quality to solve unambiguously the protein "motions".