Serial Millisecond Crystallography at the Advanced Photon Source

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The scarcity of XFEL facilities severely limits the use of serial femtosecond crystallography (SFX) while synchrotron sources are becoming viable options as a real alternative for serial millisecond crystallography (SMX) experiments. As a result, the number of SMX experiments is rapidly growing and, so far, ten experiments have been reported. Here, we present the first injector-based SMX experiments carried out at a U.S. synchrotron source, the Advanced Photon Source (APS). These experiments were conducted at the GM/CA 23-ID-D beamline. Micro-crystals of a wide variety of proteins including lysozyme, thaumatin, PSII, phycocyanin, human adenosine A_{2A} receptor (A_{2A}AR), beta-2 adrenergic receptor (β_2AR), KDO8PS, FLPP3, and Proteinase K were screened. The 1 - 15 µm crystals were delivered to the beam suspended in lipidic cubic phase, agarose or a high molecular weight PEG (MW=8,000,000), using a high viscosity injector. For each protein target, tens to hundreds of thousands of diffraction patterns were collected by a Pilatus 6M detector in shutterless mode at a repetition rate of 10 Hz, using a photon energy of 12 keV and 10 µm diameter (FWHM) beam size. In-house hit-finding software developed at APS and SFX data-reduction and analysis software suites, Cheetah and CrystFEL, enabled efficient SMX data monitoring, reduction and processing. Although hits were found for almost all proteins tested, the best diffracting crystals were from the A_{2A}AR, proteinase K, phycocyanin, FLPP3, thaumatin, and lysozyme with hit rates of 3.0%, 4.2%, 5.0%, 11.6%, 15.1%, and 34.2%, respectively, and corresponding indexing rates of 36.0% (5287 indexed patterns out of 14,711), 18% (817 indexed patterns out of 4,497), 23.1% (1,826 indexed out of 7,912), 23.6% (3,157 indexed out of 13,383), 3.3% (417 indexed out of 12,443), 15.0% (18,648 indexed out of 124,800), respectively. The structures of the A_{2A}AR, phycocyanin, FLPP3 and lysozyme were determined to 3.2 Å, 3.1 Å, 3.0 Å, and 2.2 Å resolution, respectively, demonstrating the feasibility of serial data collection at the APS using 1 – 15 µm crystals of small proteins. In all experiments, less than 60 µL of protein/carrier material were used for this study, which is far below the volumes used with liquid and LCP injectors in serial experiments at LCLS. Our results clearly demonstrate that SMX is feasible at the APS with microcrystals. The planned APS-Upgrade will increase the intensity in micro focused beams by at least two orders of magnitude enabling SMX for larger macromolecules.