Microsecond time-resolved X-ray scattering by utilizing MHz repetition rate at second-generation XFELs

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Detecting microsecond structural perturbations in biomolecules is crucial in various scientific fields such as biology, chemistry, and medicine. Given the prevalence of micro-millisecond scale reactions in biological systems, accurately monitoring and comprehending the associated chemistry is crucial. In this study, we demonstrate the utility of MHz repetition rates at X-ray free-electron lasers (XFELs) in generating microsecond time-series of protein scattering data. These time-resolved datasets exhibit remarkably low noise levels, reaching as low as 0.001%. To illustrate the potency of our approach, we investigated the Light-Oxygen-Voltage (LOV) domain from oats, a photosensory domain responsive to blue-light illumination. Upon blue-light illumination, the domain undergoes a conformational change resulting in the unfolding of its C-terminal helix, known as Jα. Utilizing our method, we gained novel mechanistic insights into the unfolding process within this photosensory domain, revealing a previously unseen intermediate state. We demonstrated that after 300 microseconds, most of the Jα-helix is unfolded by interpreting the scattering using AlphaFold predictions. Our time-resolved acquisition strategy offers a straightforward implementation and broad applicability for directly observing structural dynamics in numerous biochemical processes.