Filming protein structural dynamics in the crystalline and solution phases by time-resolved X-ray diffraction

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Recent years have witnessed the birth of picosecond (ps) pump-probe X-ray diffraction and scattering techniques [1-5]. We have employed these to study structural dynamics and spatiotemporal kinetics of many molecular systems including diatomic molecules, haloalkanes, organometallic complexes and protein molecules over timescales from ps to milliseconds. In this talk, its application to protein structural dynamics will be emphasized and picosecond time-resolved X-ray crystallography and liquidography (solution scattering) to study spatiotemporal reaction dynamics of proteins in single crystals [1] and solutions [2-5] will be presented. X-ray crystallography, the major structural tool to determine 3D structures of proteins, can be extended to time-resolved X-ray crystallography with a laser-excitation and X-ray-probe scheme, and all the atomic positions in a protein during its biological function can be tracked [1]. However the application of time-resolved protein crystallography has been limited to a few model systems with reversible photocycles due to the stringent prerequisites such as highly-ordered and radiation-resistant single crystals and crystal packing constraints might hinder biologically relevant motions. These problems can be overcome by applying time-resolved X-ray diffraction directly to protein solutions rather than protein single crystals [4]. To emphasize that structural information can be obtained from the liquid phase, this time-resolved X-ray solution scattering technique is named time-resolved X-ray liquidography (TRXL) in analogy to time-resolved X-ray crystallography where the structural information of reaction intermediates is obtained from the crystalline phase [3]. We demonstrate tracking of protein's structural changes in solution using TRXL [2-4]. By providing insights into the structural dynamics of proteins functioning in their natural environment, TRXL complements and extends results obtained with time-resolved spectroscopy and X-ray crystallography. The typical time resolution of TRXL has been limited to ~100 ps, the X-ray pulse width available from synchrotron sources, and in near future femtosecond resolution will be achieved with X-ray free electron lasers.

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References

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