Infrared Laser-Induced Temperature-Jump: A General Perturbation Method for Studying Protein Dynamics with Time-Resolved X-ray Scattering and Diffraction

M.C. Thompson[1], B.A. Barad[2], H.S. Cho[3], F. Schotte[3], A. M. Wolff[2], R.G. Sierra[4], A. Gonzalez[5], A.S. Brewster[6], I.D. Young[6], H. Demirchi[7], S.J. Kim[1], E.M. Poss[8], A. Sali[1], N.K. Sauter[7], H. van den Bedem[5,7], P.A. Anfinrud[3], J.S. Fraser[1]

1. Department of Bioengineering and Therapeutic Sciences, University of California, San Francisco, San Francisco, USA.
2. Graduate Program in Biophysics, University of California, San Francisco, San Francisco, USA.
3. Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, USA.
4. Linac Coherent Light Source, SLAC National Accelerator Laboratory, Menlo Park, USA.
5. Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory, Menlo Park, USA.
6. Lawrence Berkeley National Laboratory, Berkeley, USA.
7. Biosciences Division, SLAC National Accelerator Laboratory, Menlo Park, USA.
8. Graduate Program in Chemistry and Chemical Biology, University of California, San Francisco, San Francisco, USA.

Time-resolved X-ray scattering and diffraction are among the most information-rich experimental techniques in structural biology. To date, systems that have been successfully studied are those in which a protein conformational change is coupled to excitation of a photoactive ligand molecule, because the conformational change can be initiated with an ultrafast laser pulse. Unfortunately, the number of proteins that undergo photochemistry as part of their functional cycle is small, and there is a fundamental need to develop generalized methods that can be used to synchronously excite conformational transitions in *any* protein molecule, even in the absence of specific photochemistry. A recent “multi-temperature” crystallographic study of a model enzyme, cyclophilin A (CypA) demonstrated that temperature perturbation is an effective way to experimentally manipulate the conformational ensemble of a crystalline protein. Our current goal is to develop time-resolved X-ray experiments that utilize laser-induced temperature-jump (T-jump) excitation methods to synchronize conformational dynamics. Initial SAXS/WAXS experiments demonstrate that even modest T-jumps produce a measurable change in X-ray scattering by the protein, and allow us to develop a kinetic model for how the X-ray scattering signal changes following the IR laser pulse. We are now developing crystallographic T-jump experiments using the serial femtosecond crystallography technique at the LCLS. Because laser T-jump methods exploit photochemistry of the solvent, and not the protein molecules, we hope they will be universally applicable as a tool for studying protein dynamics at both synchrotron and XFEL light sources.