Turning Up the Heat on Dynamic Proteins: Observing molecular motion in real time with temperature-jump X-ray crystallography

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The importance of dynamics for protein function is widely appreciated; however, it remains challenging to understand, in atomic detail, how a molecule's biological activity is enabled by the physical coupling of its conformational fluctuations across varied length and time scales. Toward this end, time-resolved X-ray crystallography, in which fast X-ray pulses are used to measure structural changes of macromolecules in real time, is a powerful tool for studying dynamics because it provides simultaneous structural and kinetic data. Unfortunately, its widespread use in structural biology has been limited by a major technical hurdle: in order to observe dynamic behavior from an ensemble-averaged experimental measurement, it is necessary to synchronize conformational changes for a significant fraction of molecules in the sample. Recently, we have been able to overcome this challenge by exploiting the temperature-sensitivity of protein conformational ensembles. Specifically, we performed temperature-jump crystallography, in which a pulsed infrared laser was used to rapidly heat crystallized proteins, initiating conformational dynamics that were subsequently monitored in real time using ultrafast X-ray pulses from a free-electron laser. Our experiments captured signatures of functional motions in lysozyme, which offer new insight into the catalytic cycle of this well-studied enzyme and validate the use of temperature-jump as a universal perturbation method for time-resolved crystallographic studies of protein dynamics.