

## Investigating enzyme mechanisms by multidimensional crystallography

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Functional characterization of proteins requires linking structure and dynamics, but traditional X-ray crystallography provides only static snapshots. Serial time-resolved crystallography enables direct visualization of structural changes over time, including internal motions and solvent interactions. We developed fixed-target approaches such as “Hit And REturn”(HARE) and reaction initiation strategies using piezo droplet injectors. The “Liquid Application Method for time-resolved Analysis”(LAMA) further broadens applicability to systems not triggered by light. In addition, environmental control allows temperature variation from 7 °C to 70 °C, enabling multi-dimensional experiments. These advances permit direct observation of ligand binding, intermediates, and conformational changes, as demonstrated by tracking glucose-to-fructose conversion in Xylose isomerase across both temperature and time. Together, these methods expand the toolkit for time-resolved crystallography, opening the way to mechanistic insights into enzyme dynamics, allostery, and solvent networks.

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