

MS06-1-5 Protein dynamics probed by time-resolved crystallography on the second to hour time scale
#MS06-1-5

N. Caramello ¹, S. Engilberge ¹, S. Aumonier ², D. Von Stetten ³, G. Gotthard ⁴, G.A. Leonard ¹, C. Mueller-Dieckmann ¹, A. Royant ⁵

¹Structural Biology Group, European Synchrotron Radiation Facility - Grenoble (France), ²Photon Science Division – Laboratory for Macromolecules and Bioimaging (LSB), Paul Scherrer Institut - Villigen (Switzerland), ³European Molecular Biology Laboratory (EMBL) c/o DESY - Hamburg (Germany), ⁴Laboratory of Biomolecular Research, Biology and Chemistry Division, Paul Scherrer Institute - Villigen (Switzerland), ⁵Univ. Grenoble Alpes, CNRS, CEA, Institut de Biologie Structurale (IBS) - Grenoble (France)

Abstract

Over the last decade, the development of serial crystallography (SX) has rejuvenated room temperature macromolecular crystallography (RTMX) and led to technical and methodological breakthroughs ¹. Time-resolved SX usually addresses phenomena occurring on the picosecond to second time scale, but RTMX can be applied to study protein dynamics on slower time scales. We have applied RTMX to study the relaxation of a photostationary equilibrium built within a crystal of the LOV2 domain of phototropin 2 from *Arabidopsis thaliana*, whose photoadduct decays on the minute time scale ². We have monitored its relaxation in the dark by recording 1 s data sets using an Eiger X 4M detector, as a follow-up to a study on the population build-up of the same photoadduct ³. We could observe bond breakage in the photoadduct using both *in crystallo* UV-vis absorption spectroscopy and X-ray crystallography with similar decay time constants. Surprisingly, the return to the ground state of the chromophore is followed by additional protein rearrangements, which slow down the full recovery of the protein ground state. Our work demonstrates the possibility of performing time-resolved protein crystallography experiments from a small number of crystals using standard beamline equipment at synchrotrons for phenomena occurring on the second to hour time scale.

References

1. Schlichting, I. Serial femtosecond crystallography: the first five years. *IUCrJ* 2, 246–255 (2015).
2. Iuliano, J. N. et al. Unraveling the Mechanism of a LOV Domain Optogenetic Sensor: A Glutamine Lever Induces Unfolding of the J α Helix. *ACS Chem. Biol.* 15, 2752–2765 (2020).
3. Aumonier, S. et al. Millisecond time-resolved serial oscillation crystallography of a blue-light photoreceptor at a synchrotron. *IUCrJ* 7, 728–736 (2020).