

## Recent advances in time-resolved crystallography

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Protein conformational changes control the pathways of biochemical reactions within living cells. Time-resolved X-ray crystallography has been developed over three decades to visualize time-dependent structural changes as they occur within protein crystals (1). When successful, high-resolution X-ray diffraction data recorded during the course of a biochemical reaction can provide unique structural insight that is not accessible with other biophysical techniques. Serial crystallography, which was first developed at x-ray free-electron lasers (XFELs) (2), is now routinely used for time-resolved diffraction studies of macromolecules (3) at both XFEL and synchrotron radiation facilities. In this presentation I will review recent progress in the field of time-resolved X-ray diffraction studies of macromolecules. I will discuss the important ideas underpinning any time-resolved diffraction study, and how current advances should make it possible for others to enter the field. While aiming for an overview, where appropriate I will use work I contributed to on bacteriorhodopsin (4, 5) and a photosynthetic reaction centre (6, 7) to illustrate conceptual or methodological ideas. I will argue that the field is already enjoying an unprecedented period of growth as more systems become accessible to time-resolved diffraction.

1. G. Branden, R. Neutze, Advances and challenges in time-resolved macromolecular crystallography. *Science* **373**, (2021).
2. H. N. Chapman *et al.*, Femtosecond X-ray protein nanocrystallography. *Nature* **470**, 73-77 (2011).
3. J. Tenboer *et al.*, Time-resolved serial crystallography captures high-resolution intermediates of photoactive yellow protein. *Science* **346**, 1242-1246 (2014).
4. P. Nogly *et al.*, Retinal isomerization in bacteriorhodopsin captured by a femtosecond x-ray laser. *Science* **361**, eaat0094 (2018).
5. E. Nango *et al.*, A three-dimensional movie of structural changes in bacteriorhodopsin. *Science* **354**, 1552-1557 (2016).
6. R. Dods *et al.*, Ultrafast structural changes within a photosynthetic reaction centre. *Nature* **589**, 310-314 (2021).
7. A. B. Wohri *et al.*, Light-induced structural changes in a photosynthetic reaction center caught by Laue diffraction. *Science* **328**, 630-633 (2010).