

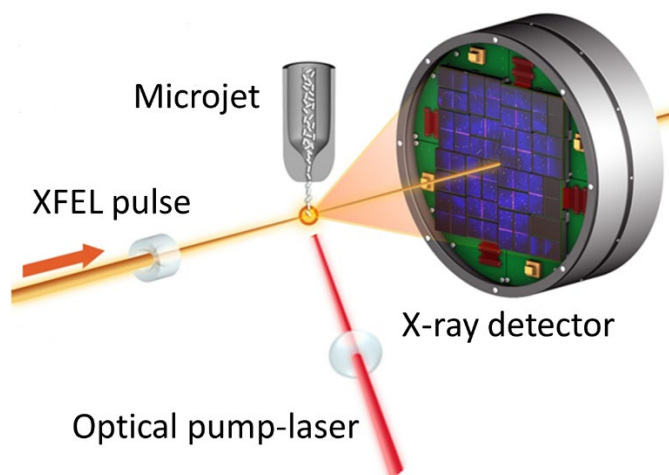
# Time-resolved X-ray diffraction studies of enzymatic reactions

Richard Neutze

<sup>1</sup>*Department of Chemistry and Molecular Biology, University of Gothenburg*

*richard.neutze@gu.se*

Time-resolved X-ray crystallography allows time-dependent structural changes to be visualized at atomic-resolution as they evolve within protein crystals over a time-domain from sub-picoseconds to seconds [1]. Serial crystallography [2] is now routinely used for time-resolved X-ray diffraction studies of macromolecules at X-ray free electron laser and synchrotron radiation facilities. As the field grows, biological reactions that are not naturally light sensitive will be increasingly studied using time-resolved serial crystallography. In this presentation I will outline the development of time-resolved X-ray crystallography and in particular the growth of the field since the first proof-of-principle demonstration of time-resolved serial femtosecond X-ray crystallography [3]. Structural results from the light-driven proton pump bacteriorhodopsin [4] will be used to illustrate what is possible with these methods as well as discussing some controversies in the field [5].



**Figure 1.** Schematic of a pump-probe time-resolved serial X-ray crystallography setup used at an X-ray free electron laser. Schematic taken from Reference [1].

- [1] Branden, G. & Neutze, R. (2021). *Science* **373**, eaba0954; Khusainov, G., Standfuss, J. & Weinert, T. (2024). *Struct Dyn* **11**, 020901.
- [2] Chapman, H. N., *et al.* (2011). *Nature* **470**, 73-77.
- [3] Tenboer, J., *et al.* (2014). *Science* **346**, 1242-1246.
- [4] Nango, E., *et al.* (2016). *Science* **354**, 1552-1557; Nogly, P. *et al.* (2018). *Science* **361**, eaat0094; Bertrand, Q. *et al.* (2024). *Nat Commun* **15**, 10278.
- [5] Barends, T. R. *et al.* (2024). *Nature* **626**, 905-911; Neutze, R. & Miller, R. J. D. (2024). *Nature* **626**, 720-722.