Complementarity of

cryo-trapping and time-resolved protein X-ray crystallography

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Kinetic X-ray crystallography permits the structural characterization of macromolecular conformational changes along a reaction pathway at the atomic level of spatial resolution. After triggering the biological reaction within a macromolecular crystal, functionally relevant conformational changes are either arrested by flash-cooling the crystal, allowing characterization of the structure by conventional cryo macromolecular crystallography (MX; Weik & Colletier, 2010), or followed in real time by time-resolved crystallography at room temperature (Caramello & Royant, 2024). The temporal resolution of the latter is limited to 100 ps if carried out in the form of Laue crystallography at synchrotrons. The advent of X-ray free electron lasers (XFELs) has pushed the resolution to the sub-ps regime, allowing ultrafast changes to be studied by time-resolved serial femtosecond crystallography (TR-SFX).

Using the vitamin-B12 dependent photoreceptor CarH (Poddar *et al*., 2023) as an example, we will illustrate the power of combining cryo-trapping MX and *in crystallo* UV-vis spectroscopy (von Stetten *et al*., 2015) at the ESRF BM07-FIP2 beamline with TR-SFX at XFELs (unpublished).

1) Weik, M. and J.-P. Colletier (2010). "Temperature-dependent macromolecular X-ray crystallography." Acta Crystallographica Section D 66(4): 437-446.

2) Caramello, N. and A. Royant (2024). "From femtoseconds to minutes: time-resolved macromolecular crystallography at XFELs and synchrotrons." Acta Crystallographica Section D 80(2).

3) von Stetten, D., T. Giraud, P. Carpentier, F. Sever, M. Terrien, F. Dobias, D. H. Juers, D. Flot, C. Mueller-Dieckmann, G. A. Leonard, D. de Sanctis and A. Royant (2015). "In crystallo optical spectroscopy (icOS) as a complementary tool on the macromolecular crystallography beamlines of the ESRF." Acta crystallographica. Section D, Biological crystallography 71(Pt 1): 15-26.

4) Poddar, H., R. Rios-Santacruz, D. J. Heyes, M. Shanmugam, A. Brookfield, L. O. Johannissen, C. W. Levy, L. N. Jeffreys, S. Zhang, M. Sakuma, J.-P. Colletier, S. Hay, G. Schirò, M. Weik, N. S. Scrutton and D. Leys (2023). "Redox driven B12-ligand switch drives CarH photoresponse." Nature Communications 14(1): 5082.