MS09 Enzymology

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Kinetic X-ray crystallography to watch proteins in action

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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-crystallography (intermediate trapping), or followed in real time by time-resolved crystallography at room temperature. 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. We will illustrate the cryo-trapping approach by following product release in an insect carboxylesterase that hydrolyses organophosphates [1]. Radiolytic cleavage of the bond between the active-site serine and the organophosphate is observed at 150 K but not at 100 K. The increased solvent and protein mobility at 150 K is thus required to allow for product release and accompanying protein conformational changes to occur. Time-resolved crystallography will be exemplified with the study of ultra-fast photoswitching in a fluorescent protein from a non-fluorescent (*off*) to a fluorescent (*on*) state further to excitation by a light flash [2]. Our consortium (see author list of [2]) has employed time-resolved serial femtosecond crystallography at an XFEL to identify the transient structure of the photoswitchable fluorescent protein rsEGFP2 in its excited state, and to observe its chromophore in a twisted conformation, midway between the stable configurations of the *on* and *off* states. This observation has been confirmed by simulations and has allowed to rationally design a mutant with a two-fold increased photoswitching quantum yield.

References:

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[2] Coquelle N, Sliwa M, Woodhouse J, Schirò G, Adam V, Aquila A, Barends TRM, Boutet S, Byrdin M, Carbajo S, De la Mora E, Doak RB, Feliks M, Fieschi F, Foucar L, Guillon V, Hilpert M, Hunter MS, Jakobs S, Koglin JE, Kovacsova G, Lane TJ, Lévy B, Liang M, Nass K, Ridard J, Robinson JS, Roome CM, Ruckebusch C, Seaberg M, Thepaut M, Cammarata M, Demachy I, Field M, Shoeman RL, Bourgeois D, Colletier J-P, Schlichting I, Weik M (2018) Chromophore twisting in the excited state of a photoswitchable fluorescent protein captured by time-resolved serial femtosecond crystallography. Nature Chemistry 10: 31-37

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