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Mix-and-probe serial femtosecond crystallography enables the visualisation of an enzyme:substrate complex at room temperature

Wednesday, 7 September 2022 12:00 (20 minutes)

Understanding the basis of enzyme catalysis at the molecular level has been a fundamental goal of biochemistry and structural biology. The "recording"of an enzymatic reaction as it happens represents a significant challenge because many of the enzyme:substrate/transition-state/product complexes are transients, making them invisible to standard crystallographic techniques. X-ray free-electron lasers produce ultra-fast femtosecond X-ray pulses *ca.* 10_{12} times more intense than standard synchrotron radiation. Nonetheless, it is critical to develop injector/mixing devices to exploit XFEL capabilities to visualise enzymatic reactions.

In this talk, we will present the application of the concentric-flow electrokinetic injector **[1]** to carry out *mix-and-probe* serial femtosecond crystallography, which allows us to mix unbound enzyme microcrystals with natural substrates and inject them into the path of femtosecond XFEL pulses to obtain structural information of retaining glycosyltransferases. Also, we will present our results using standard synchrotron radiation and quick-soaking of natural substrates into unbound enzyme crystals **[2]** to trap structural snapshots of glycosyltransferases **[3,4]** which allow us to propose a common substrate-assisted S_Ni-type mechanism for all retaining GTs **[4]**.

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2. Albesa-Jové D, Cifuente JO, Trastoy B, Guerin ME: **Quick-soaking of crystals reveals unprecedented insights into the catalytic mechanism of glycosyltransferases**. In *Methods in Enzymology*. Academic Press; 2019:261–279.
3. Albesa-Jové D, Mendoza F, Rodrigo-Unzueta A, Gomollón-Bel F, Cifuente JO, Urresti S, Comino N, Gómez H, Romero-García J, Lluch JM, et al.: **A Native Ternary Complex Trapped in a Crystal Reveals the Catalytic Mechanism of a Retaining Glycosyltransferase**. *Angew Chemie Int Ed* 2015, 54:9898–9902.
4. Albesa-Jové*D, Sainz-Polo MÁ, Marina A, Guerin* ME: **Structural Snapshots of α-1,3-Galactosyltransferase with Native Substrates: Insight into the Catalytic Mechanism of Retaining Glycosyltransferases**. *Angew Chemie Int Ed* 2017, 56:14853–14857. (*corresponding author)

Would you like to participate in the Poster Prize competition?

No

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