# Structural investigation of the reaction mechanism of a glucuronyl esterase using time-resolved serial crystallography

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We combine traditional rotational X-ray crystallography with time-resolved serial X-ray crystallography to investigate the reaction mechanism of glucuronyl esterases (GEs), enzymes of significant interest for industrial biomass conversion. GEs contribute to plant cell wall deconstruction by cleaving covalent linkages between lignin and carbohydrates. Efficient disruption of these linkages is essential for the full utilization of lignocellulosic biomass as a renewable resource in biorefineries (1).

Our study focuses on the bacterial GE *Ot*CE15A from *Opitutus terrae*, which has previously been characterized both biochemically and structurally (2). In contrast to fungal GEs, several bacterial GEs exhibit broader substrate specificity, showing activity on both glucuronic and galacturonic acid-based model substrates. Structural insights into how these enzymes process different substrates are therefore of high interest for optimizing enzyme cocktails used in industrial biomass degradation.

We have obtained static cryogenic structures from crystals soaked with different model substrates across a range of pH conditions, aiming to modulate reaction rates. To further control and resolve reaction progression, we performed time-resolved serial crystallography experiments (3) using the hit-and-return (HARE) method in combination with the liquid application method for time-resolved analysis (LAMA) at P14-2, PETRA III (DESY, Hamburg) (4), capturing substrate turnover on the millisecond timescale. The cryogenic structures reveal multiple catalytic intermediate steps that together with the time-resolved serial data provide a more detailed view of the GE reaction mechanism of different substrates.

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