# Exploring serial crystallography for capturing Michaelis complexes of a glucuronyl esterase involved in lignocellulose degradation

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We live in a contradictory society where, on the one hand, we face devastating environmental consequences caused by excessive use of non-renewable fossil resources, but at the same time, we are heavily dependent on them and are depleting our reservoirs. Research into environmentally friendly and renewable resources is therefore of utmost importance, and a great interest is focused on biomass resources, where lignocellulosic resources possess a huge potential. However, the structure of lignocellulose is complex and difficult to degrade, which poses a significant challenge for utilizing the feedstock in biorefineries. One of the main reasons why lignocellulose is hard to degrade is the presence of covalent linkages connecting lignin with cell wall polysaccharides. Glucuronyl esterases (GEs) are enzymes able to cleave such linkages between lignin and glucuronoxylans. Thanks to this, GEs could potentially contribute with important activities when processing biomass with biological pretreatment methods (1).

We are studying a GE from the carbohydrate esterase family 15, produced in the bacteria *Phocaeicola vulgatus* (*Pv*CE15). Previous studies of the enzyme have identified it to be located in a polysaccharide utilization loci appearing to target pectin, in contrast to xylan as would have been expected (2). Furthermore, the enzyme has shown catalytic activity on galacturonate model substrates proposed to mimic covalent linkages between lignin and pectin. These observations have given rise to questions regarding the enzyme's biological role and whether it might be involved in degrading substrates other than xylan. Structural studies of the enzyme, using traditional cryo X-ray crystallography, have shown galacturonate binding in a distinct conformation compared to glucuronate. However, attempts to capture Michaelis complexes of the enzyme binding model substrates prior to hydrolyzing the ester bond have so far not succeeded. Obtaining the structure of Michaelis complexes may aid in gaining a better understanding of the enzyme and its mechanism of action, as a step in improving biological pretreatment methods. We aim to investigate if the Michaelis complex can be obtained using time-resolved serial crystallography. So far, we have been able to obtain well-diffracting microcrystals suitable for serial crystallography where we have obtained the very first room-temperature structure of the protein at 2.1 Å.

1. Abolore, R. S., Jaiswal, S., & Jaiswal, A. K. (2023). Green and sustainable pretreatment methods for cellulose extraction from lignocellulosic biomass and its applications: A review. Carbohydrate Polymer Technologies and Applications, 100396.
2. Seveso, A., Mazurkewich, S., Banerjee, S., Poulsen, J. C. N., Lo Leggio, L., & Larsbrink, J. (2024). Polysaccharide utilization loci from Bacteroidota encode CE15 enzymes with possible roles in cleaving pectin-lignin bonds. Applied and Environmental Microbiology, 90(1), e01768-23.