ESRF	**Experiment title:** Structural and functional architecture of a catalytic center of a maize cytokinin glucoside-specific beta-glucosidase	**Experiment number**: **MX- 98**
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Report:

Glycoside hydrolases (GH) catalyze the selective hydrolysis of glycosidic bonds within oligosaccharides and polysaccharides or between carbohydrates and non-carbohydrate moieties. Based on amino acidsequence similarities, GHs are currently classified into 112 families. In plants, GHs are involved in the metabolism of cell wall polysaccharides, biosynthesis and remodulation of glycans, mobilization of storage reserves, defense, symbiosis, secondary metabolism, glycolipid metabolism and signaling. Plant β-glucosidases belonging to family 1 retaining GHs are a widespread group of enzymes that hydrolyze a broad variety of aryl- and alkyl-β-D-glucosides as well as glucosides with only carbohydrate moieties. There is considerable interest in plant β-glucosidases, because they are involved in diverse biological processes, ranging from developmental regulation, for example, activation of the plant hormones cytokinins, through cell wall degradation to pathogen defense reactions.

A maize β-glucosidase, Zm-p60.1, a member of the GH1 family, has been shown to release active cytokinins from their O- and N3-glucosides, and thus has implicated roles in the regulation of maize seedling development. The enzyme has been located in plastids, and its accumulation in chloroplasts and plastids of transgenic tobacco has been shown to perturb the cytokinin metabolic network.

Analysis of three-dimensional structures has provided indications that the enzymes' specificity toward substrates with aryl aglycones is conferred by the aromatic aglycone system stacking with W373, and van der Waals interactions with edges of F193, F200, and F461 located opposite W373 in a slot-like aglycone-binding site [1].

With an aim for better understanding of atomic mechanism of Zm-p60.1, we had collected in experiment MX-98 five complete datasets - two with point mutants of Zm-p60.1 and three with complexes of Zm-p60.1 with different substrates.

The best data had beed obtained with point mutant E186Q. Crystal with cell parameters a=97.36, b=112.59, c=99.26Å, α=90°, β=103.22°, γ=90° (= different crystal system than original crystals of WT described in [1]) diffracted up to 1.7Å resolution and resulting structure has beed refined up to R=15.4%, Rfree=20.1%.. The results of MX-98 experiment (together with the results of preceding and sequent Zm-p60.1 experiments) were used mainly as an input and validation during our extensive investigation of Zm-p60.1 mutants by methods of molecular modeling [2].

[1] Zouhar, J., Vévodová, J., Marek, J., Damborský, J., Su, X.-D. & Brzobohatý, B.: Insights into the Functional Architecture of the Catalytic Center of a Maize β-Glucosidase Zm-p60.1. Plant Physiol. 127, 973-985 (2001).

[2] Dopitová, R., Mazura, P., Janda, L., Chaloupková, R., Jeřábek, P., Damborský, J., Filipi, T., Kiran, N. S. & Brzobohatý, B.: Functional analysis of the aglycone-binding site of the maize beta-glucosidase Zm-p60.1. *FEBS J.* **275**, 6123-6135 (2008).