Microscale thermophoresis as a powerful tool for screening glycosyltransferases involved in cell wall biosynthesis.

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Project Goals: Develop the fundamental understanding of cell wall biology and Develop tools to facilitate bioenergy crop improvement.

Identification and characterization of key enzymes associated with cell wall biosynthesis and modification is fundamental to gain insights into cell wall dynamics. However, the challenge is that activity assay of glycosyltransferase is very low throughput and acceptor substrates are generally not available. Here we optimized and validated microscale thermophoresis (MST) to achieve high throughput screening for glycosyltransferase substrates. MST is a powerful new method for the quantitative analysis of protein-ligand interactions with low sample consumption. The technique is based on the motion of molecules along local temperature gradients, measured by fluorescence changes. We optimized the methods to allow the determination of binding affinity by MST without purification of the target protein from the cell lysate. The application of this MST method to beta-1,4-galactosyltransferase AtGALS1 validated the capability of substrate screening. We also expanded the application to detect binding of AtGALS1 to its acceptor in presence of UDP. Furthermore, we used the method in combination with activity assays to identify potential features of the poplar ortholog PtGALS1 in substrate binding and catalytic activity. For this study we used a set of PtGALS1 mutant variants that had been designed based on the crystal structure of the enzyme as determined by X-ray crystallography. An automated pipeline using automated liquid handling technique was established to facilitate the high-throughput substrate screening of glycosyltransferases in sorghum, and until now twenty sorghum GT61s were screened with potential substrates, which will enable selection of candidates for further studies and engineering.

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