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# Exploring effect of spatial geometries in plant cell wall degrading multienzymatic complexes – A case study with cellulases and a xylanase

Iker Pardo Larrabeiti<sup>1</sup>, Pierre Roblin<sup>2</sup>, David Ropartz<sup>3</sup>, Helene Rogniaux<sup>3</sup>, Mathieu Fanuel<sup>3</sup>, Bastien Annic<sup>3</sup>, Sarah Moraïs<sup>4</sup>, Ed Bayer<sup>4</sup>, Claire Dumon<sup>1</sup>, Cédric Montanier<sup>1</sup>

<sup>1</sup> TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France

<sup>2</sup> LGC, Université Paul Sabatier, UMR 5503, Toulouse, France

<sup>3</sup> BBS platform, Mass Spectrometry lab, Nantes, France.

<sup>4</sup> Ben-Gurion University, Beer-Sheva, Israel

[cedric.montanier@insa-toulouse.fr](mailto:cedric.montanier@insa-toulouse.fr)

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To address the intricate complexity of plant cell walls (PCWs), anaerobic microorganisms using polysaccharides as carbon source produce an extensive range of glycoside hydrolases (GHs) organized in a multienzymatic complex: the cellulosome. Despite the designer cellulosome approach developed to study the effect of the spatial arrangement of GHs on enzymatic activities [1], the synergies and the spatial position of the different enzymes within the complex are still poorly understood due to its high flexibility. One approach to address this issue is to lock the spatial proximity between the GHs. To achieve this, we employ the Biomolecular Welding tool composed of two small proteins, Jo and In [2]. These proteins spontaneously form an intramolecular isopeptidic bond and offer a unique approach to geometrically freeze the spatial conformation between several GHs (Fig.1). It has effectively been used to demonstrate the impact of the spatial conformation between a xylanase and a  $\beta$ -xylosidase towards xylan hydrolysis [3].

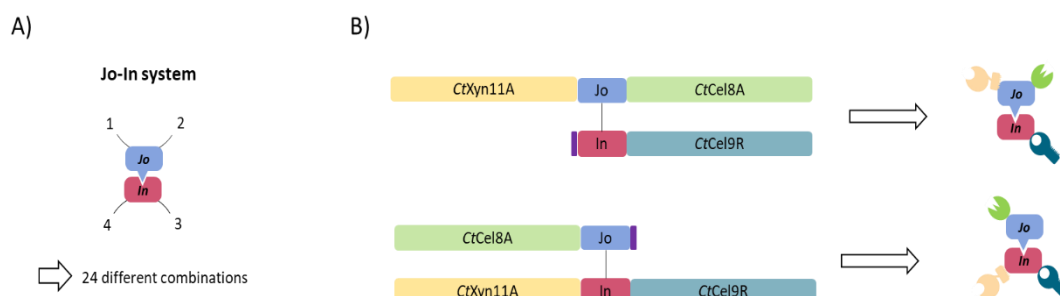


Figure 1A: Jo-In system extremities available for fusion (24 different combinations can be achieved using 3 enzymes). 1B: Exemplification of two distinct complexes resulting from Jo-In system fusion.

In this study, we propose to focus on three cellulosomal GHs from *C. thermocellum* (CtCel8A, CtCel9R, and CtXyn11A) to produce various combinations of multienzymatic complexes using the Jo-In system. Based on our hypothesis that the relative position of the enzymes within the complex is crucial, we aim to correlate PCWs degradation and product profile over time with the complex topology. The full set of the different possible organizations of the three enzymes was produced and purified. In this work we compared the enzymatic activities of all the complexes assessed on cellulose, xylan, and wheat bran (natural substrate) to those of the free enzymes in solution. The most promising complexes underwent an in-depth characterization through Small-angle X-ray scattering, while the product profile was analyzed via mass spectrometry. With this we explore how the spatial topology of multienzymatic complexes is of importance in biocatalysis.

[1] Fierobe H-P. et al. JBC 2001 276,24

[2] Bonnet J. et al. Sci. Rep. 2017 7:43564

[3] Enjalbert T. et al. *Int. J. Mol. Sci.* 2020 21, 4360