Investigating the effect of the spatial topology of multimodular GHs on an insoluble substrate

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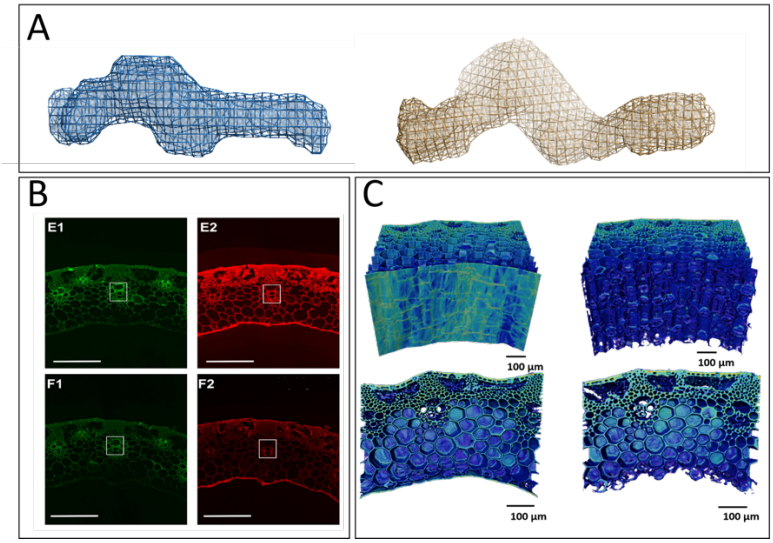
The wide diversity of carbohydrate-active enzymes (CAZymes) reflects the equally wide diversity in the composition and chemical bonds of the plant cell wall polysaccharides. This diversity is also reflected in the different strategies developed by microorganisms to circumvent the recalcitrance of these substrates to biological degradation. These aspects have been studied extensively for decades. CAZymes are also very diverse in terms of architecture, from the simplest to the most complex, such as the cellulosome. Such organisations have been shown to be important for catalytic function. However, the role of spatial topology and distances between the different domains of individual enzymes acting on a complex and insoluble substrate is underestimated. We propose a systematic approach to investigate this question, focusing on glycoside hydrolases (GHs). Our approach is based on two small proteins, Jo and In, which spontaneously form an intramolecular isopeptide bond and, incidentally, provide an original means of orienting GHs [1]. Chimeric multimodular GHs were produced and purified, and their structure in solution was solved by Small Angle X-rays Scattering (Fig.1A) [2]. In addition to their activity towards soluble and insoluble substrates, differences in the targeting of multimodular GHs in wheat straw were assessed *in situ*, monitored by immunological labelling (Fig.1B). We also demonstrated that X-ray microtomography is particularly well-suited to reveal the modification of the plant cell walls within the sample, at different stages of the enzymatic deconstruction (Fig.1C) [3].



A) SAXS model of chimeric GHs; B) Double immunofluorescence of paraffin-embedded wheat straw serial sections; C) Images from X-ray microtomographyA) S

Bibliographic references:

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