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A tailored and non-altering enzyme immobilization approach to explore plant biomass deconstruction

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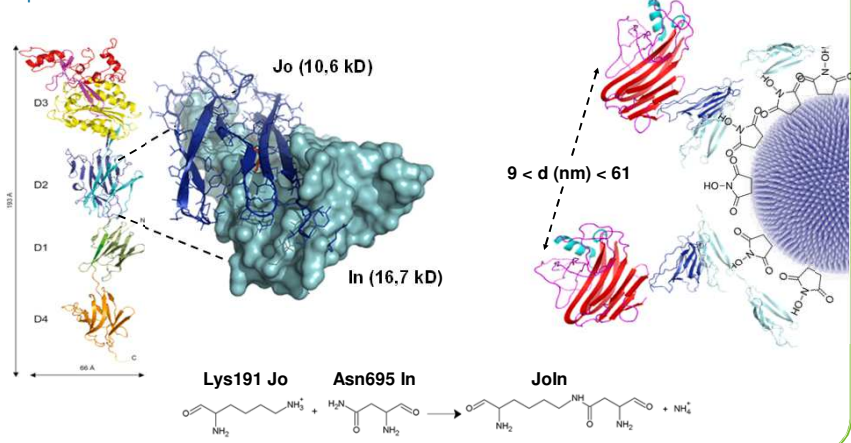
Context and Objective

In Nature, the plant-based organic carbon contained within plant cell walls is mainly recycled by the action of cellulolytic microorganisms, such as bacteria and fungi, which produce complex arrays of cell wall-degrading enzymes. Many of the enzymes that degrade plant cell wall polysaccharides are modular proteins, which contain single or multiple copies of both catalytic domain(s) and CBM(s). Cellulolytic microorganisms developed a couple of strategies to break down the complex plant cell wall; secrete free enzymes into their surroundings or produce large multi-component cell-bound structures, which harbour several enzymes displaying complementary activities. In both case, substrate is attacked by different enzymes acting together at different regions, and much more than sum of individual different enzymatic activities, synergism drives the efficiency of such system. However, spatial proximity between enzymes and its impact over synergism is rarely investigated.

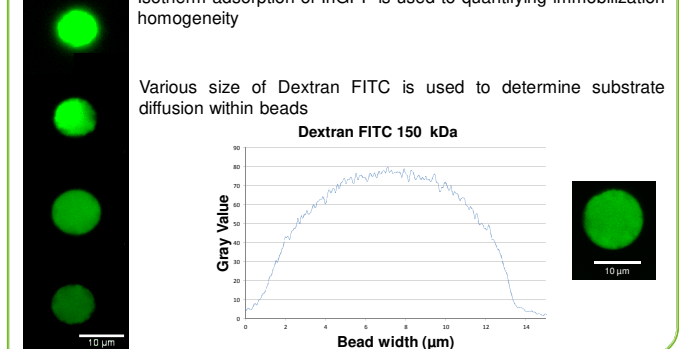
Here we report an original way to easily and precisely control distance between molecules of the xylanase Xyn11A from *Neocallimastix patriciarum*¹ through a covalent immobilization on a solid support and we analysed the impact of such immobilization according to the size of the products released.

Results

1 Precise control of the distance between immobilized enzymes using Jo and In recombinant proteins^{2,3}.



3 Homogenous immobilization and substrate diffusion using fluorescence



2 Distance between immobilized molecules and kinetics.

Distance between molecules of InNpXyn11A from *Neocallimastix patriciarum* is controlled and measured:

- commercial beads
- decreasing amount of enzymes
- Langmuire surface adsorption (BET)

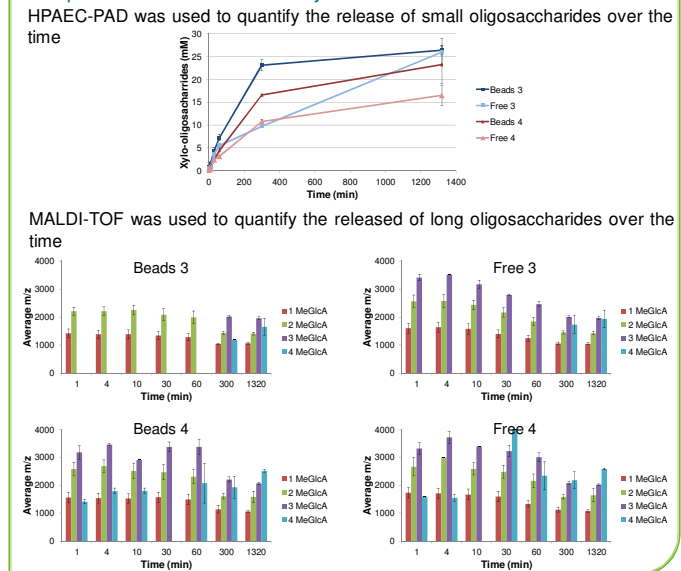
	InNpXyn11A on beads d (nm)
Beads 1	8.6
Beads 2	11.1
Beads 3	21.7
Beads 4	28.4
Beads 5	39.0
Beads 6	60.7

Effect of the immobilization is investigated using small and long substrate and compared to the equivalent amount of free enzymes

	pNP-Xylotriside*		Beechwood xylan**				
	InNpXyn11A	Beads 1	InNpXyn11A	Beads 1	Beads 3	Beads 5	Beads 6
K_m	2,7±0,04 mM	2,0±0,2 mM	1,1 ± 0,07 (mg/ml)	3,6 ± 0,4 (mg/ml)	3,6 ± 0,4 (mg/ml)	2,1 (mg/ml)	1,6 (mg/ml)
k_{cat} (min ⁻¹)	215,4±8,2	150,4±0,7	3,3 10 ⁴ ± 3,6 10 ³	8,9 10 ³ ± 9,6 10 ²	2,2 10 ⁴ ± 2,6 10 ³	3,8 10 ⁴	3,2 10 ⁴
k_{cat}/K_m (ml/min/mg)	78,8±4,3	73,84±5,5	3,0 10 ⁴ ± 1,2 10 ³	2,4 10 ³ ± 7,1 10 ¹	6,1 10 ³ ± 9,5 10 ¹	1,8 10 ⁴	2,4 10 ⁴

50 mM Tris/HCl pH7 5.1 mg/mL, BSA at 37°C. * 5 mM pNP-Xyloside or ** 2% Beechwood xylan.

4 Comparing hydrolysis products of xylan released from beads and equivalent amount of free enzymes.



Conclusions

Here we report an original way to easily and precisely control distance between molecules of the xylanase Xyn11A from *Neocallimastix patriciarum* through Joln covalent immobilization on a solid support. Immobilization did not modify the enzymatic activity on chromogenic substrate and Beechwood xylan. Distance is the only parameter. This approach allowed us to control distance between enzymes from 8 to 60 nm and compare the kinetic to the equivalent amount of free enzymes. Product profile for both immobilized and free enzymes were assessed by HPAEC-PAD and MALDI-TOF. A distance of 20 nm seems to increase dramatically the production of small oligosaccharides probably because of an homogeneous production of medium sized oligomers.

