Immobilized enzymes at work: when surface density matters Context and Objective

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Immobilized enzymes at work:
when surface density matters

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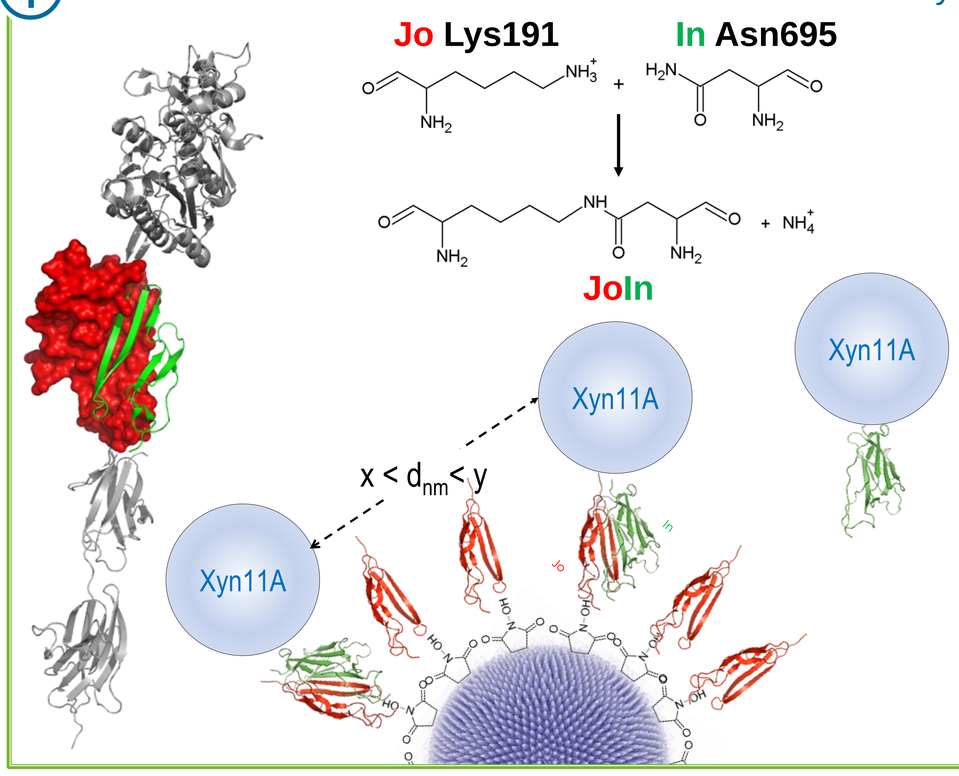
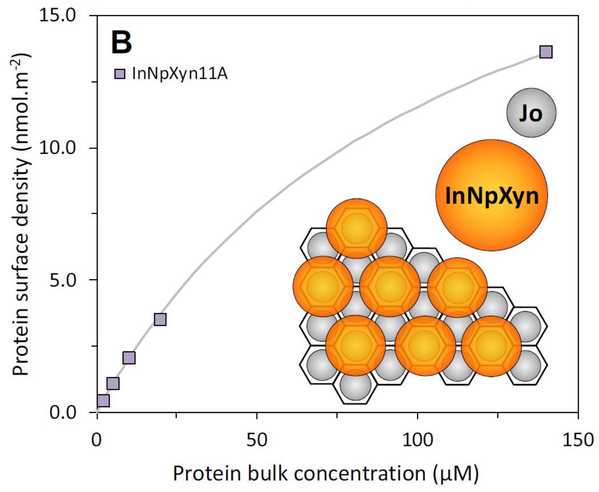
Context and Objective

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). Thus, 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. Furthermore, in the case of the cellulosome, a large multi-component cell-bound structures, enzymes are localized at close distance to each-other. The benefits of this spatial proximity on the efficiency of the enzymatic reaction are still poorly understood. We investigate this question by using an in-house developed system, Jo-In, where enzymes are immobilized with controlled densities - therefore distances - that can be controlled precisely. The enzyme used is a xylanase that participates to the hydrolysis of plant cell wall polymers, the Xyn11A from *Neocallimastix patriciarum*. **Our approach preserved the intrinsic activity of the enzyme, making the density of grafting the only parameters that is tuned[1].**

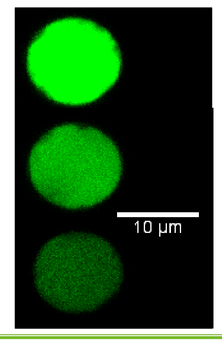
Results

① Precise control of the distance between immobilized enzymes using Jo and In recombinant proteins[2,3].

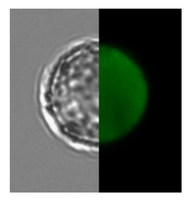




Beads identification number	InNpXyn11A surface density (nmol.m^{-2})	Average center-to-center distance between adjacent InNpXyn11A (nm)	
		Random Poisson process	Hexagonal lattice
0*	21.2	4.4	9.5
1	13.6	5.5	11.9
2	3.5	10.8	23.3
3	2.1	14.2	30.5
4	1.1	19.5	41.9
5	0.5	30.0	64.4



Confocal microsopy on beads coated with In-GFP



Confocal microscopy using fluorescent long polymers

② Immobilization does not affect enzyme activity; only distance does.

Specific activity using small chromogenic substrate *p*NP-X_3

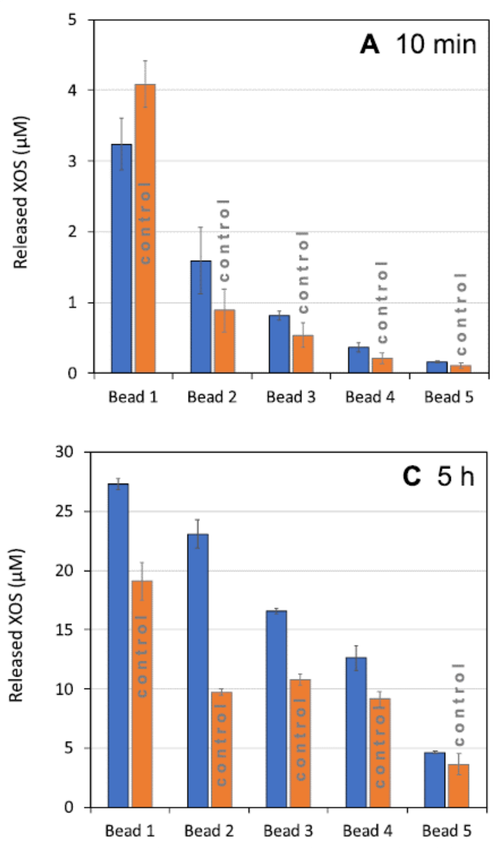
Specific activity SA (µmol.min^{-1}.mg^{-1})			
Immobilized enzymes			Free enzymes
Beads	(A) at constant beads volume fraction = 0.3%	(B) at constant total concentration of InNpXyn11A in solution = 4.47 mg/L	(C) at various InNpXyn11A concentrations
1	2.12 (4.47)[a]	2.14 (0.3%)[b]	2.29 (4.47)[c]
2	2.88 (1.15)[a]	2.14 (0.7%)[b]	2.48 (1.15)[c]
3	2.52 (0.67)[a]	2.10 (1.3%)[b]	2.45 (0.67)[c]
4	2.56 (0.35)[a]	2.12 (2.7%)[b]	2.38 (0.35)[c]
5	2.19 (0.15)[a]	2.10 (5.9%)[b]	2.14 (0.15)[c]

[a] the equivalent concentration of enzyme that corresponds to 0.3% of beads volume fraction,as expressed in mg/L, [b] the volume fraction of beads that corresponds to 4.47 mg/L of In*Np*Xyn11A, [c] the concentration of free In*Np*Xyn11A in solution, as expressed in mg/L

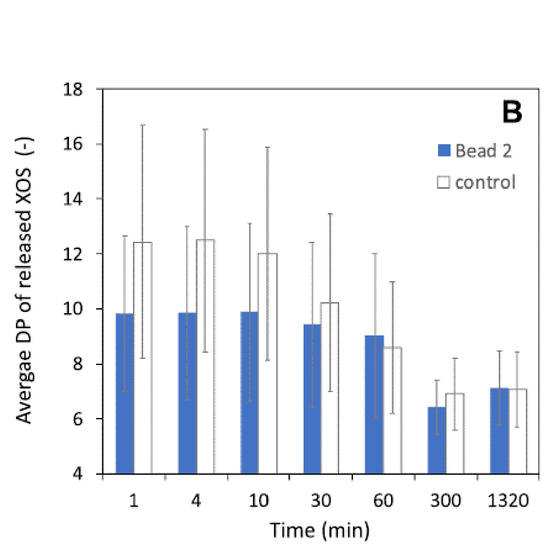
Kinetic parameters using beecwodd xylan as long chain polymer

	Free InNpXyn11A	Beads 0	Beads 2	Beads 4	Beads 5
Surface density (nmol.m^{-2})	—	21.2	3.5	1.1	0.5
K_m (mg.mL^{-1})	1.8 ± 0.7	3.6 ± 0.5	3.6 ± 0.5	2.6	2.1
k_{cat} (10^3 min^{-1})	46.1 ± 8.8	8.9 ± 0.9	22.4 ± 2.7	43.5	55.1
k_{cat}/K_m (10^3 min^{-1}.mg^{-1}.mL)	25.6 ± 6.5	2.5 ± 0.07	6.2 ± 9.5	16.5	26.2

③ Enzyme proximity and hydrolytic profile.



Concentration of xylooligosaccharides X_1 to X_6 determined by HPAEC-PAD



Average degree of polymerization of xylooligosaccharides (DP 3 to 30) released over the time detected by MALDI-TOF experiments (linear detector) with beads 2. The vertical bars are standard deviations and not error bars. They reflect the degree of size polydispersity of the released chains. Control experiments were performed with free enzymes in solution at equivalent concentration of beads.

Conclusions

Overall, results show that xylanase molecules can be distanced from 9.5 to 64.4 nm center-to-center. Using small 4-nitrophenyl-ß-D-xylotrioside as substrate, no modification of the kinetic parameters is observed compare to the enzyme in solution. However, when long polymer beechwood xylan is used as substrate, kinetic parameters are affected with higher density of grafting. The product profile was analyzed by HPAEC-PAD and MALDI-TOF. Data indicate that immobilized enzyme product profiles are different from those produced by the enzymes dispersed in solution; the immobilized enzymes release more short oligo-saccharides and oligomers with average DP more homogenous. Our results question the relationship between spatial proximity and synergistic effect as encountered in the cellulosome.

1 – Montanier *et* al., 2019 **2 - Izoré *et* al., 2010** **3- Bonnet *et* al., 2017**

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