Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Molecular dynamics simulations of the *Bacillus subtilis* expansin EXLX1: interaction with substrates and structural basis of the lack of activity of mutants

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Contents:

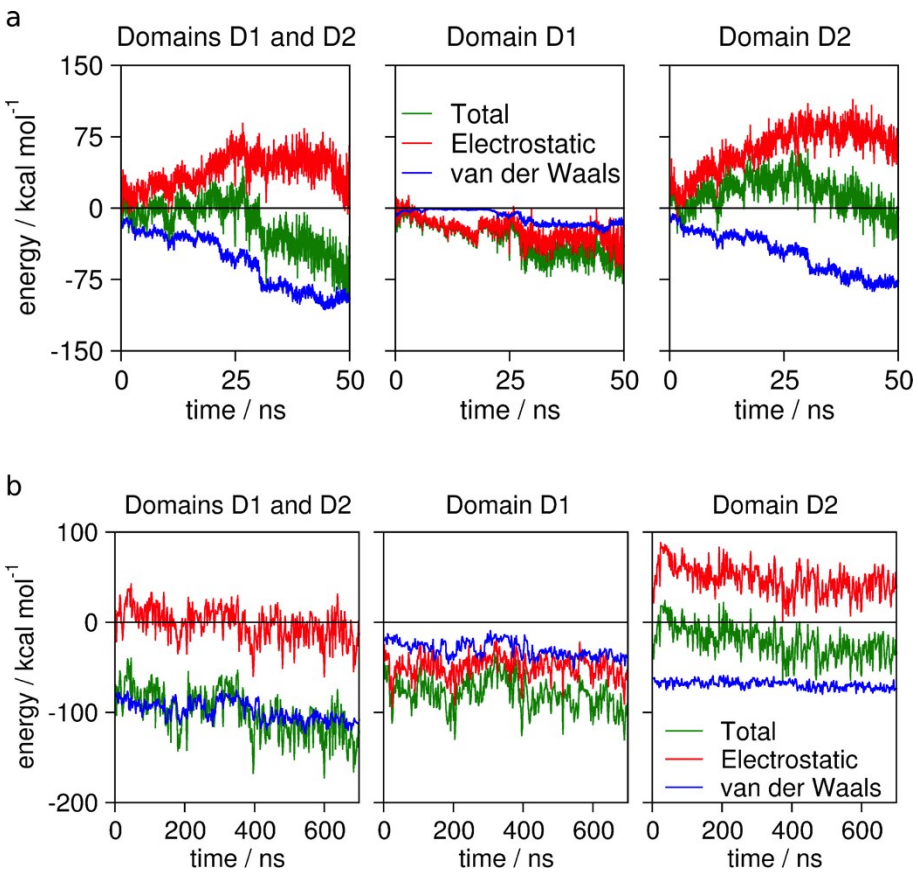

Fig. S1 Interaction energies between cellulose and the intact BsEXLX1 expansin (both domains D1 and D2; left), the domain D1 (middle) and domain D2 (right) during (a) the adsorption process in the MD2 and (b) during the 700 ns after the adsorption process. The energies fluctuate around approximately constant values after the 50-ns-long adsorption simulation, indicating that BsEXLX1 has properly accommodated on the cellulose surface.

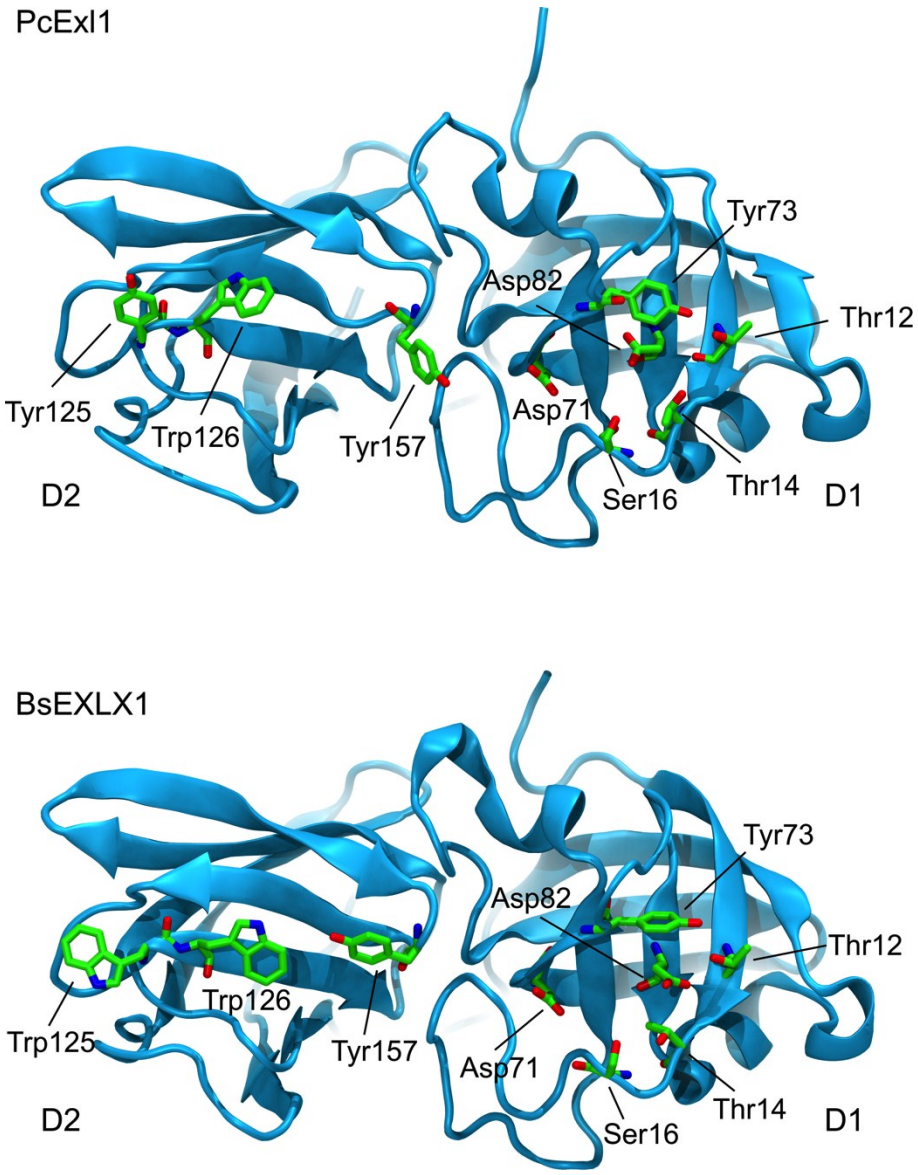

Fig. S2 Comparison between the 3D structures of *Pectobacterium carotovorum* expansin-like protein (PcExl1), obtained by homology modeling with I-TASSER[1] and of the BsEXLX1 crystallographic structure (PDB code: 3D30). These proteins exhibit 57% of sequencial identity, and share the same domain D1 binding surface and similar domain D2 binding surface. Due to the domain D1 similarity between these proteins, the effect of the mutation Asp82Ala on cellulose disrupting activity of PcExl1 – loss of activity, as measured for PcExl1 by Olarte-Lozano *et al.*[2] – is likely valid to the BsEXLX1 expansin too.

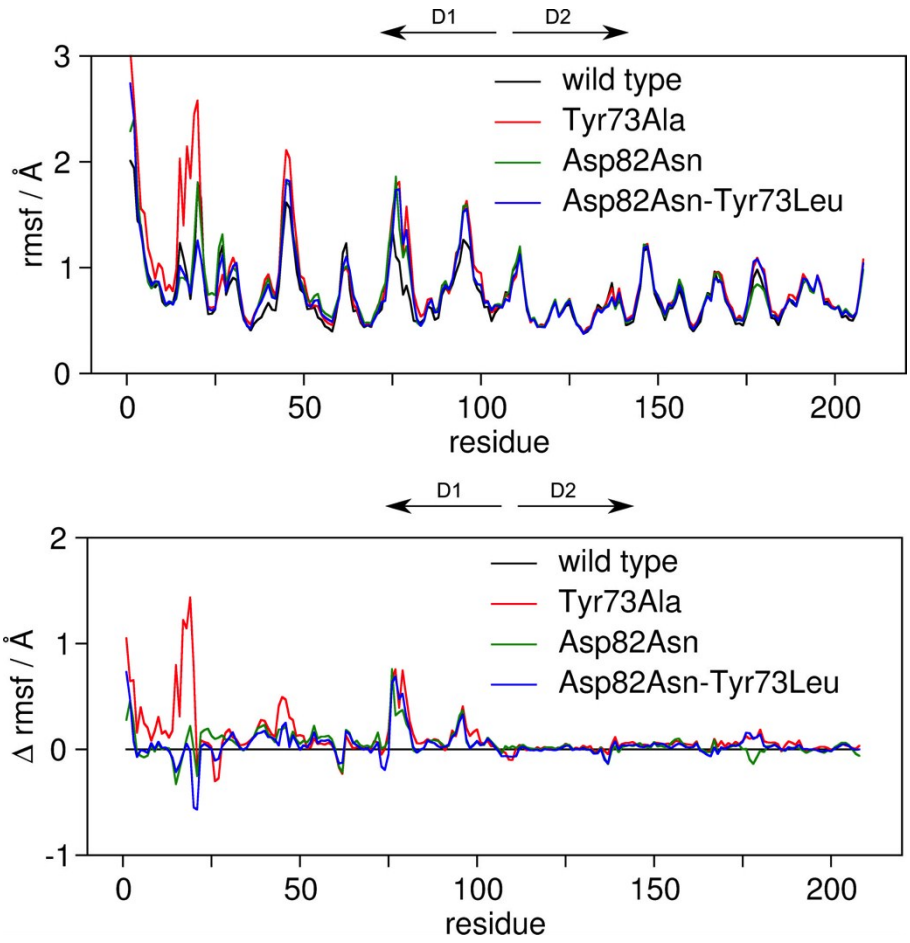

Fig. S3 Structural fluctuations of the wild type BsEXLX1 and of the mutants Asp82Asn, Tyr73Ala and Asp82Asn/Tyr73Leu. The upper panel shows the rmsf of the α-carbons and the lower panel shows the differences of the rmsf between the mutants and the wild type BsEXLX1. The rmsf's are averages taken over three independent simulations. All the mutations induce a slight increase in the fluctuations around the residue 80, but only the mutation Tyr73Ala results in major structural alterations (rmsf > 1.0 Å) in functional regions (residues Thr14 and Ser 16). The domain D2 is insensitive to the mutations in the domain D1.

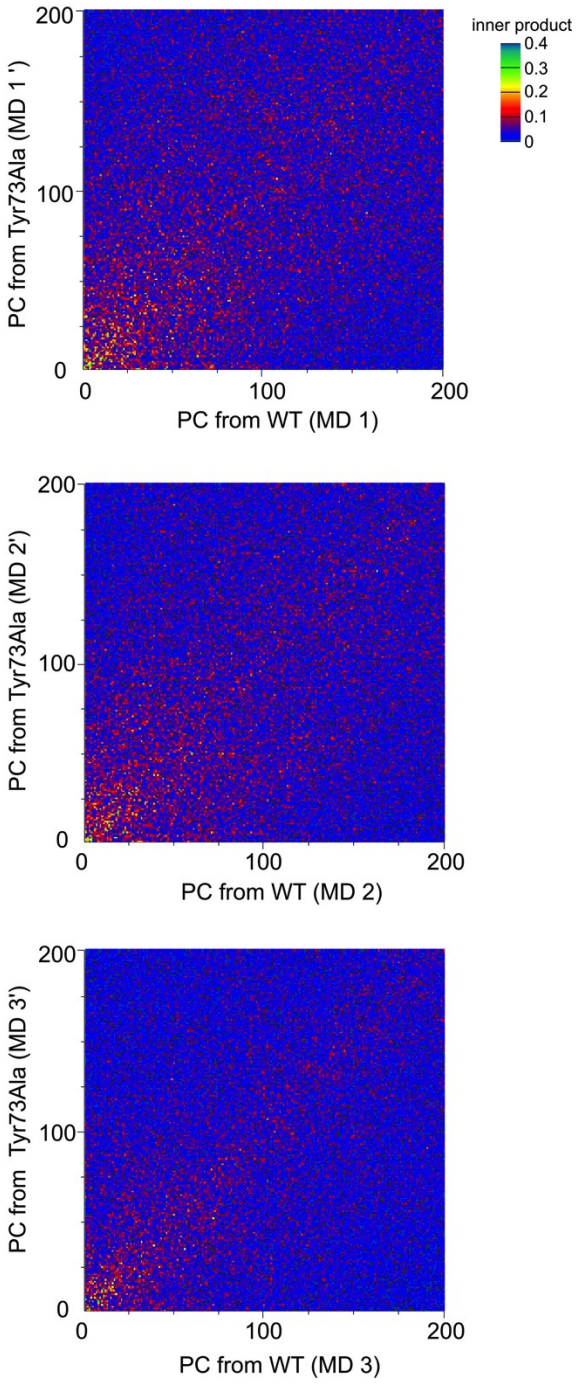

Fig. S4 Similarity between the principal components (PC) of the wild type (WT) BsEXLX1 and of the Tyr73Ala mutant, as measured by the absolute value of the inner product between each pair of the 200 first eigenvectors obtained by principal component analysis. The inner products were taken between PCs obtained from pairs of three independent simulations of each system. Simulations of WT BsEXLX1 are indicated by MD1, MD2 and MD3, and simulations of the Tyr73Ala mutant are indicated by MD1', MD2' and MD3'. The similarities between the PCs are low, as indicated by the inner products close to zero.

Table S1 Inner products between the first two principal components (PC) from the wild type (WT) and Tyr73Ala BsEXLX1. Three different pairs of independent simulations are considered. Simulations of WT BsEXLX1 are labeled MD1, MD2 and MD3. Simulations of Tyr73Ala mutant are labeled MD1', MD2' and MD3'.

PC from WT	PC from Tyr73Ala	Inner Product		
		MD1 x MD1'	MD2 x MD2'	MD3 x MD3'
1	1	-0.059	-0.371	-0.259
1	2	-0.129	0.234	-0.180
2	1	0.238	-0.193	-0.279
2	2	-0.116	-0.200	-0.219

REFERENCES

1 Y. Zhang, *BMC Bioinformatics,* 2008, **9**, 40.

2 M. Olarte-Lozano, M. A. Nuñez-Mendonza, N. Pastor, L. Segovia, J. Folch-Mallol and C. Martínez-Anaya, *Plos One,* 2014, **9**, e95638.