Supporting Information

Structural inspection and protein motions modelling of a fungal glycoside hydrolase family 18 chitinase by crystallography depicts a dynamic enzymatic mechanism

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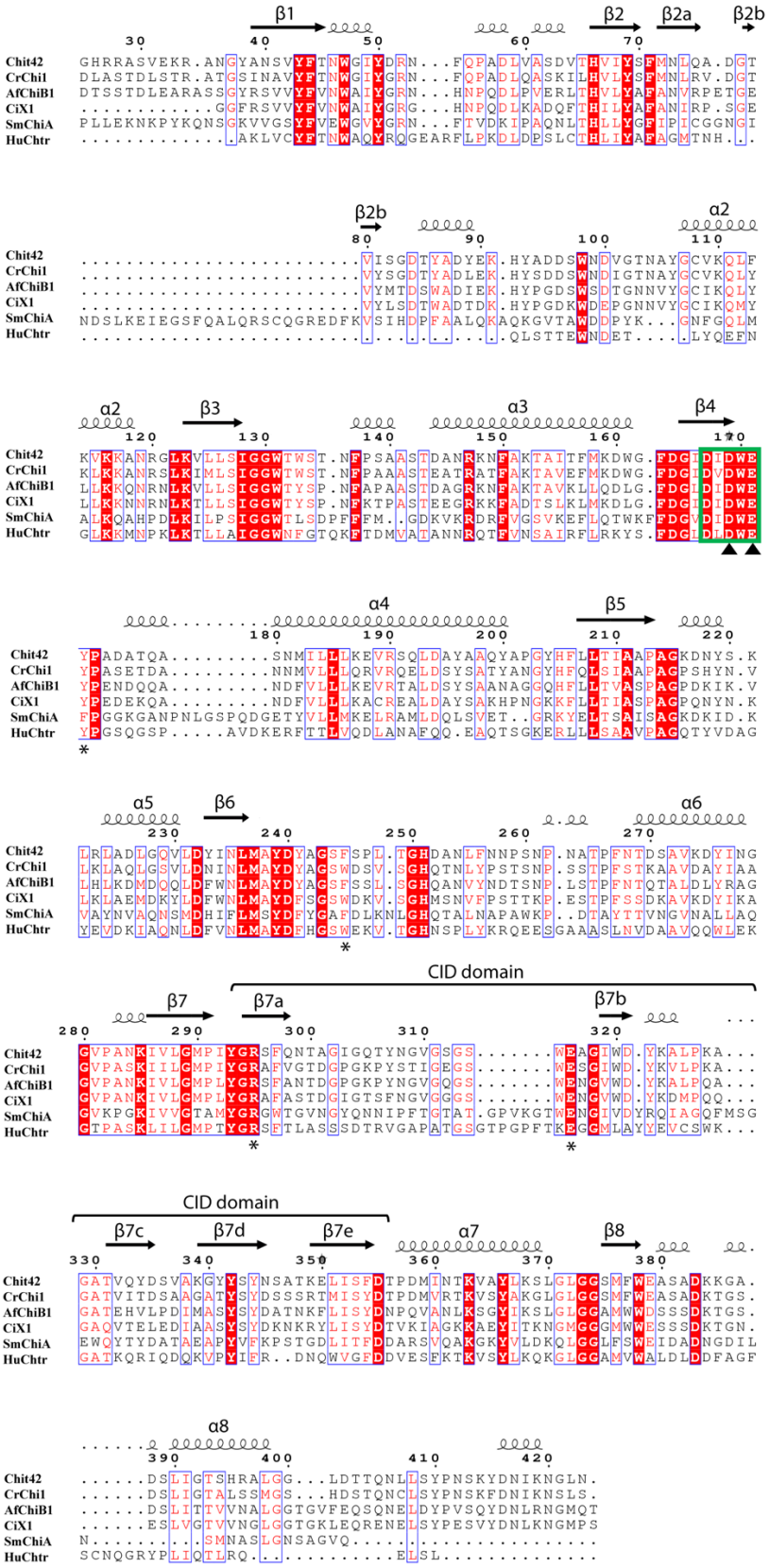


Figure S1*:* Sequence alignment of Chit42 with homologues. Sequences from the fungal chitinases from *Clonostachys roseau* (CrChi1), *Aspergillus fumigatus* (AfChiB1) and *Coccidioides immitis* (CiXi), the bacteria chitinase A from *Serratia marcescens* (SmChiA) and human chitotriosidase (HuChtr) are shown (ESPript - http://espript.ibcp.fr). DXDXE motif is boxed in green. Inverted triangles indicate catalytic residues and asterisks mark residues mutated in this work.

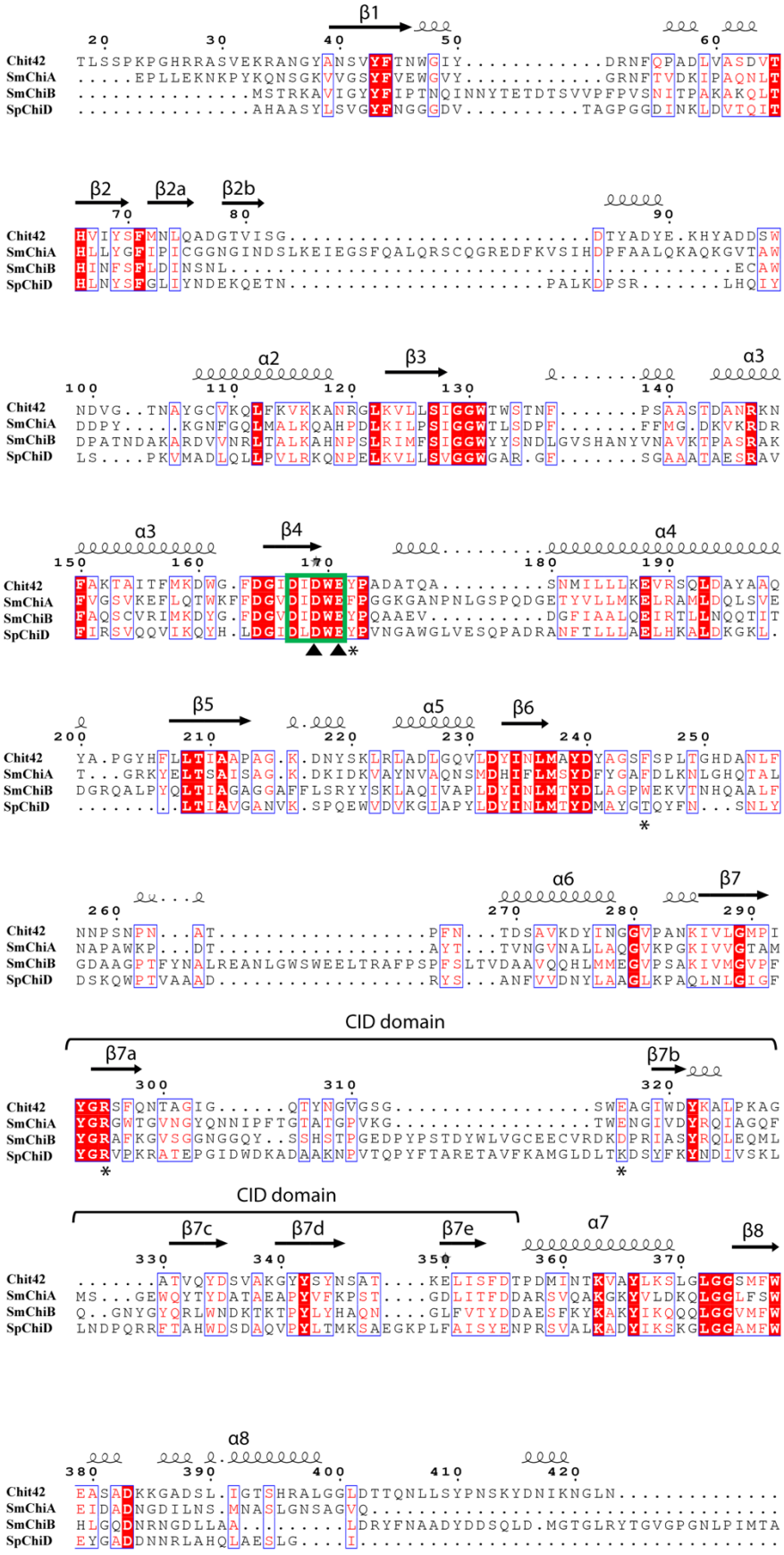


Figure S2*:* Sequence alignment of Chit42 vs bacterial chitinases. Sequences from the *Serratia marcescens* chitinase A (SmChiA), the *Serratia marcescens* chitinase B (SmChiB) and the *Serratia proteamaculans* chitinase D (SpChiD) are shown (ESPript - http://espript.ibcp.fr). DXDXE motif is boxed in green. Inverted triangles indicate catalytic residues and asterisks mark residues mutated in this work.

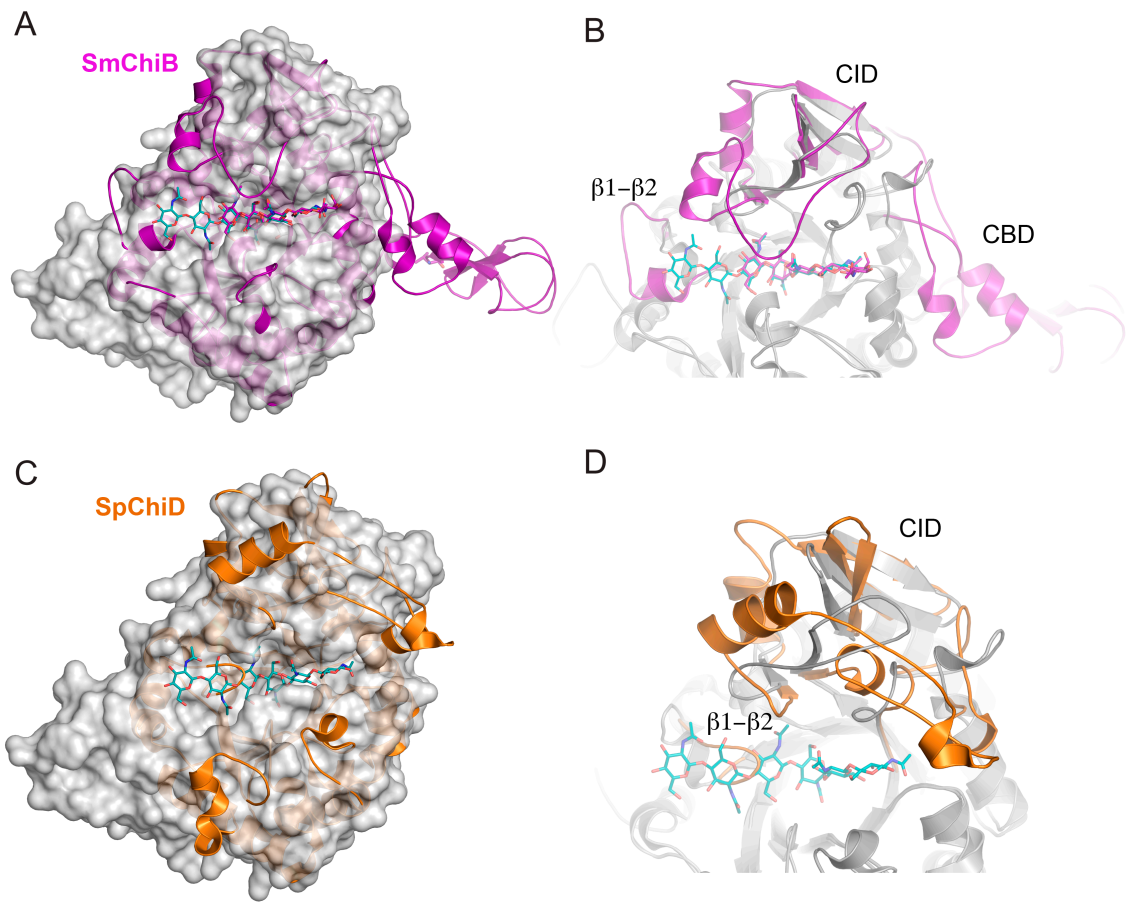
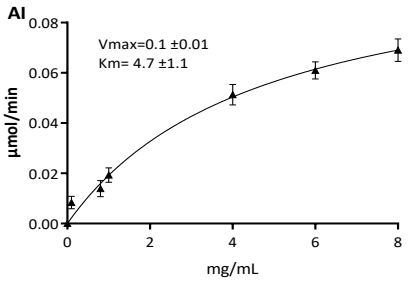
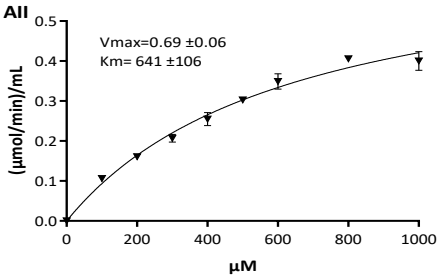
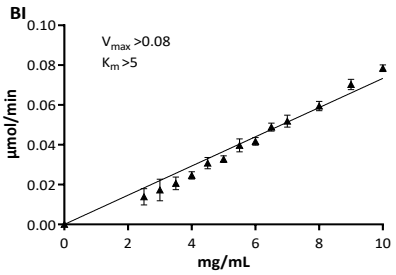
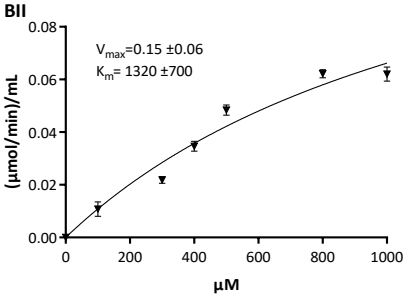


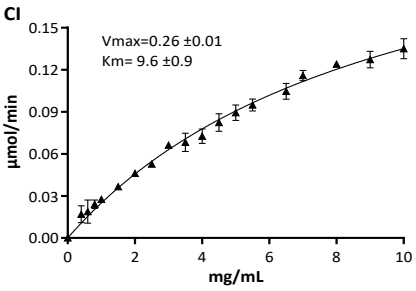
Figure S3: Structural comparison of Chit42 with bacterial chitinases. Superposition of Chit42 (gray) complexed with NAG6 (cyan) onto SmChiB complexed with NAG5 (A and B), PDB code 1E6N [**8**], and SpChiD (C and D), PDB code 4NZC [**38**]. The molecular surface of Chi42 is shown in A and C to highlight the main structural elements protruding in the bacterial enzymes. Relevant structural elements of SmChiB (B) and SpChiD (D) shaping the active site are labelled.


AI
Vmax=0.1 ±0.01
Km= 4.7 ±1.1
µmol/min
mg/mL


AII
Vmax=0.69 ±0.06
Km= 641 ±106
(µmol/min)/mL
µM


BI
Vmax >0.08
Km >5
µmol/min
mg/mL


BII
Vmax=0.15 ±0.06
Km= 1320 ±700
(µmol/min)/mL
µM


CI
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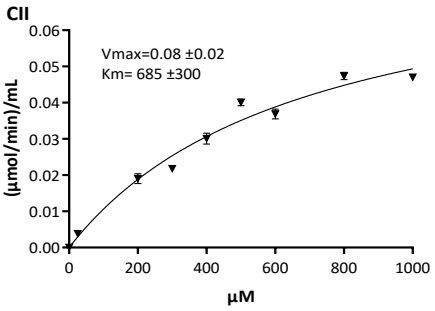
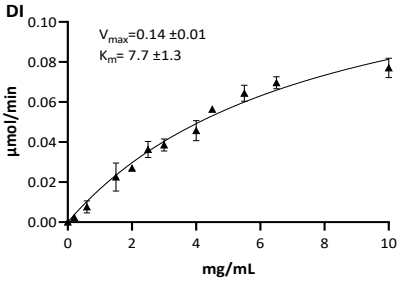
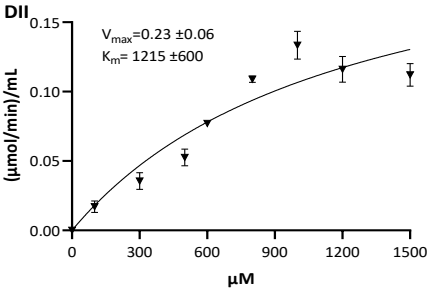
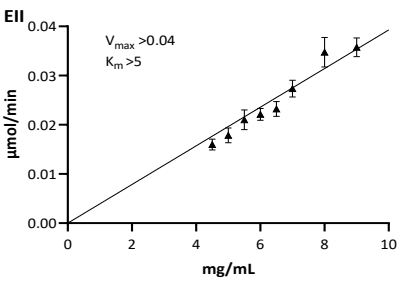
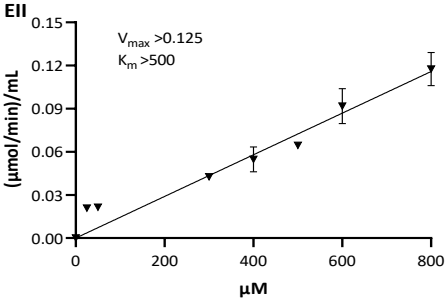
Km= 9.6 ±0.9
µmol/min
mg/mL


CII
Vmax=0.08 ±0.02
Km= 685 ±300
(µmol/min)/mL
µM


DI
Vmax=0.14 ±0.01
Km= 7.7 ±1.3
µmol/min
mg/mL


DII
Vmax=0.23 ±0.06
Km= 1215 ±600
(µmol/min)/mL
µM


EII
Vmax >0.04
Km >5
µmol/min
mg/mL


EII
Vmax >0.125
Km >500
(µmol/min)/mL
µM

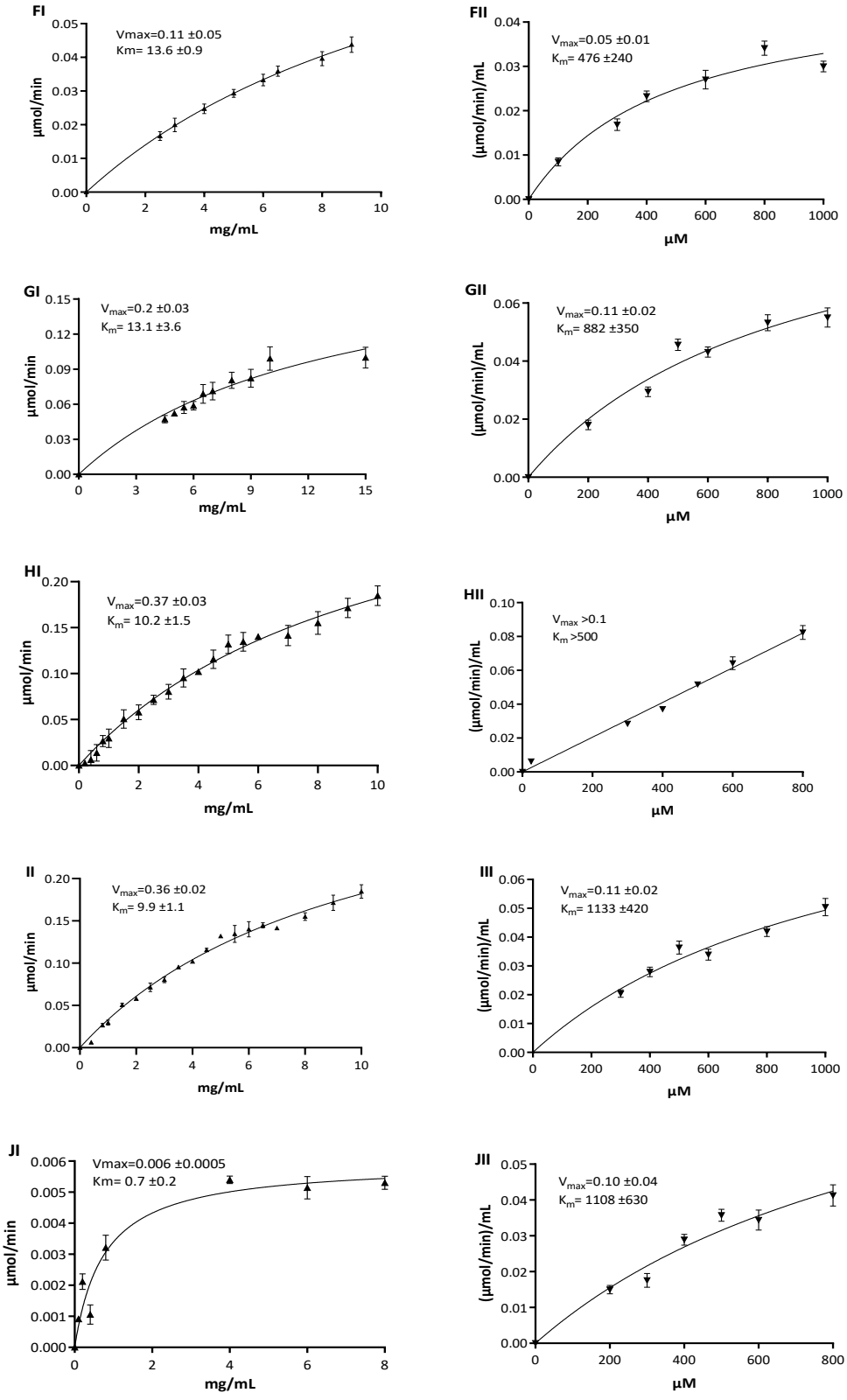


Figure S4: Michaelis–Menten Kinetics of Chit42 and its variants using colloidal chitin (I) and NAG6(II) as substrate. A to J represent WT, Y172E, Y172F, F245N, R295A, R295S, R295T, E316A, E316N, E316S respectively. Kinetic parameters, calculated by GraphPad Prism 8 software fitting the initial rate values to the Michaelis-Menten equation, are included in each panel.

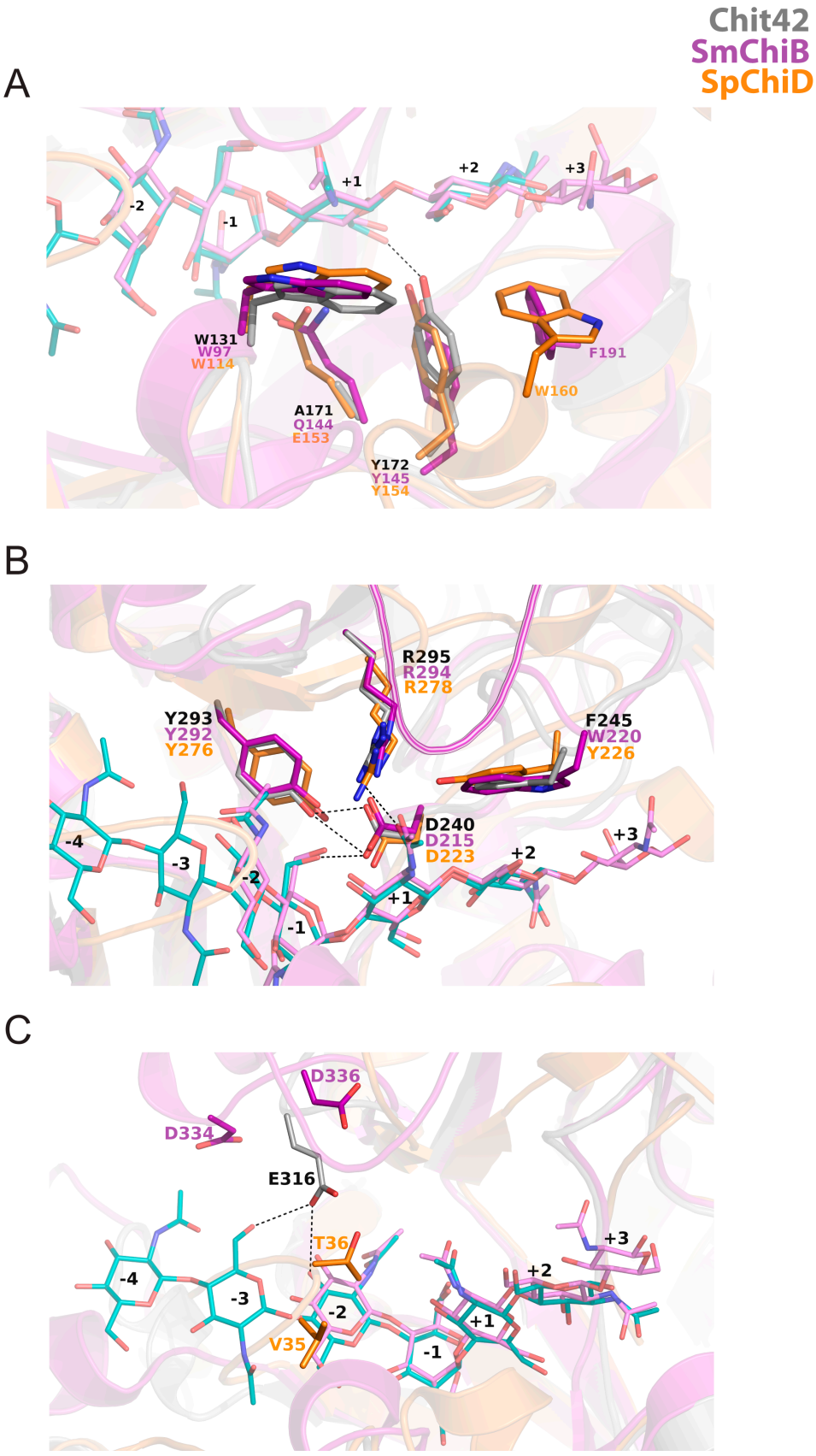


Figure S5: Relevant structural differences of Chit42 with bacterial chitinases. Detail of the superposition of Chit42 (gray) complexed with NAG6 (cyan) onto the SmChiB-NAG5 complex (magenta, PDB code 1E6N [**8**], and SpChiD (orange, PDB code 4NZC [**38**]. Main structural features at the environment of Tyr172, Arg295 and Glu316 are shown at panels A, B and C.

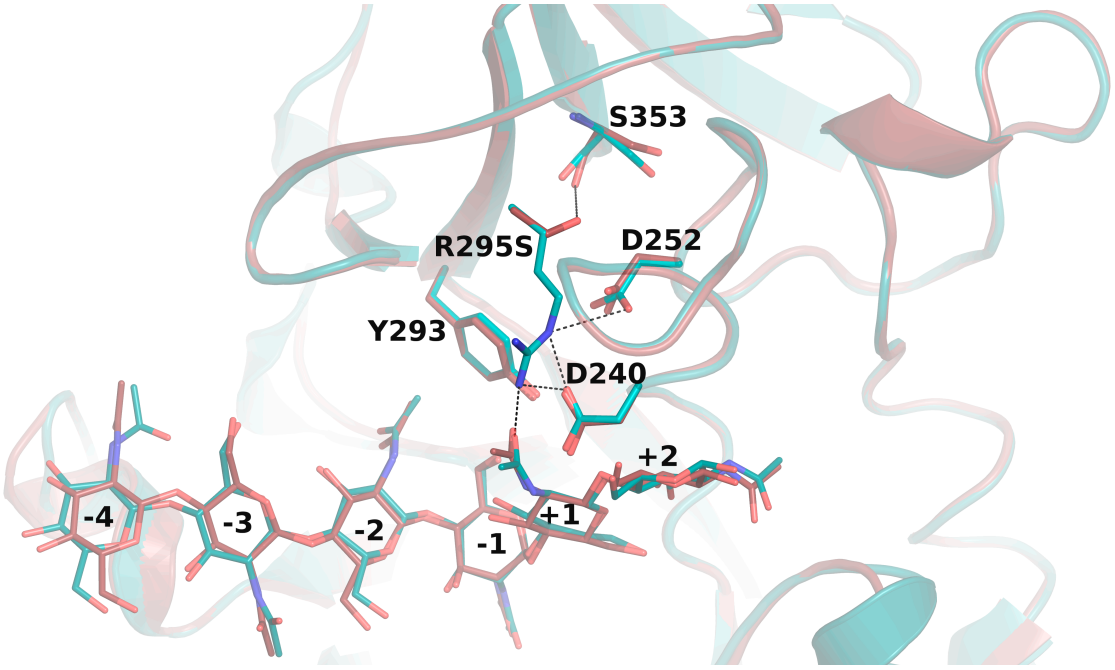


Figure S6: Model of the R295S Chit42 mutant complexed with NAG6, as calculated by MD. The molecular dynamic calculations were performed using the simple model perturbation module of Phenix [**28**] to shake up the complex model (firebrick), with the ligand position inferred from the structural superimposition onto the Chit42-NAG6 complex (cyan). The R295S mutant loses the interactions associated to the Arg295 side-chain, while the new Ser295 hydroxyl could make a polar link to Ser353 main-chain, linking two strands within the CID β-sheet. Minor conformational changes observed in some aromatics are not shown for clarity.

Table S1. Oligonucleotides designed for mutagenic PCR of Chit42**.**

(+), forward; (-), reverse. Substituted positions are in bold

Oligonucleotide	Mer	Sequence
E171A (+)	34	TATCGACTGG**GCT**TACCCTGCAGACGCCACCCAG
E171A (-)	40	CTGCAGGGTA**AGC**CCAGTCGATATCAATACCATCGAAACC
Y172E (+)	31	GACTGGGAG**GAA**CCTGCAGACGCCACCCAGG
Y172E (-)	38	GTCTGCAGG**TTC**CTCCCAGTCGATATCAATACCATCGA
Y172F (+)	31	GACTGGGAG**TTT**CCTGCAGACGCCACCCAGG
Y172F (-)	29	CGGTGAGGGGGCT**GTT**GGATCCGGCGTAG
F245N (+)	29	CTACGCCGGATCC**AAC**AGCCCCCTCACCG
F245N (-)	29	CGGTGAGGGGGCT**GTT**GGATCCGGCGTAG
R295A (+)	29	CTACGCCGGATCCAAC**AGC**CCCCTCACCG
R295A (-)	33	ATCTACGGA**GCT**TCATTCCAGAACACCGCTGGT
R295S (+)	33	CTGGAATGA**AGC**TCCGTAGATGGGCATGCCGAG
R295S (-)	33	ATCTACGGA**TCT**TCATTCCAGAACACCGCTGGT
R295T (+)	33	CTGGAATGA**AGA**TCCGTAGATGGGCATGCCGAG
R295T (-)	33	ATCTACGGA**ACT**TCATTCCAGAACACCGCTGGT
E316A (+)	33	ATCCCAGATACCGGC**AGC**CCAGCTTCCACTTCC
E316A (-)	33	GGAAGTGGAAGCTGG**GCT**GCCGGTATCTGGGAT
E316N (+)	33	ATCCCAGATACCGGC**GTT**CCAGCTTCCACTTCC
E316N (-)	33	GGAAGTGGAAGCTGG**AAC**GCCGGTATCTGGGAT
E316S (+)	43	TTGTAATCCCAGATACCGG**CAC**TCCAGCTTCCACTTCCAACAC
E316S (-)	43	GTGTTGGAAGTGGAAGCTGGA**GTG**CCGGTATCTGGGATTACAA
D169NE171A (+)	44	TGGTATTGATATC**AAC**TGG**GCT**TACCCTGCAGACGCCACCCAGG
D169NE171A (-)	42	TGCAGGGTA**GTT**CCA**AGC**GATATCAATACCATCGAAACCCCA
D169AE171A (+)	44	TGGTATTGATATC**GCT**TGG**GCT**TACCCTGCAGACGCCACCCAGG
D169AE171A (-)	42	TGCAGGGTA**AGC**CCA**AGC**GATATCAATACCATCGAAACCCCA

Table S2. Crystallographic statistics (Values in parentheses are for the high-resolution shell)

Crystal data	D169A/E171A Chit42- NAG6 soaking	D169A/E171A Chit42-NAG4/NAG2 cocrystallization	D169A/E171A Chit42-NAG4/NAG2 soaking
Space group	P 4_1 2_1 2	P 4_1 2_1 2	P 4_1 2_1 2
Unit cell parameters			
a (Å)	68.16	68.35	68.11
b (Å)	68.16	68.35	68.11
c (Å)	178.86	177.76	176.64
Data collection			
Beamline	XALOC (ALBA)	XALOC (ALBA)	XALOC (ALBA)
Temperature (K)	100	100	100
Wavelength (Å)	0.979300	0.979240	0.979300
Resolution (Å)	48-20-1.75 (1.78-1.75)	48.33-1.82 (1.86-1.82)	48.16-2.32 (2.40-2.32)
Data processing			
Total reflections	315553 (16313)	251852 (15208)	140408 (13003)
Unique reflections	43306 (2325)	38716 (2258)	16652(1651)
Multiplicity	7.3 (7.0)	6.5 (6.7)	8.4 (7.9)
Completeness (%)	99.7 (100.0)	99.9 (100.0)	90.1 (92.3)
Mean I/σ (I)	16.9 (3.7)	14.2 (4.8)	8.5 (3.3)
R_{merge} [†] (%)	8.2 (67.8)	10.7 (70.1)	18.3 (66.6)
R_{pim} [††] (%)	3.2 (27.0)	4.5 (28.6)	6.4 (24.9)
CC1/2 (%)	99.8 (87.3)	99.7 (91.7)	98.7 (77.4)
Molecules / ASU	1	1	1
Refinement			
R_{work} / R_{free} [†††] (%)	16.37/19.64	17.98/21.15	19.52/24.40
Nº of atoms/average B (Å^2)			
Protein	3032/18.17	3045/15.68	2996/24.13
Ligands	100/26.97	128/30.24	95/31.64
Water Molecules	431/30.39	393/27.30	207/25.58
All atoms	3563/19.90	3566/17.48	3298/24.44
Ramachandran plot (%)			
Favoured	97	96	96
Outliers	0	0	0
RMS deviations			
Bonds (Å)	0.0067	0.0064	0.0053
Angles (°)	1.4888	1.4764	1.4737
PDB accession codes	6YLJ	6YN4	7AKQ

[†]$R_{merge} = \sum_{hkl} \sum_{i} | I_i(hkl) - [I(hkl)] | / \sum_{hkl} \sum_{i} I_i(hkl)$, where $I_i(hkl)$ is the ith measurement of reflection hkl and [I(hkl)] is the weighted mean of all measurements.
[††]$R_{pim} = \sum_{hkl} [1/(N-1)]^{1/2} \sum_{i} | I_i(hkl) - [I(hkl)] | / \sum_{hkl} \sum_{i} I_i(hkl)$, where N is the redundancy for the hkl reflection.
[†††]R_{work} / $R_{free} = \sum_{hkl} | Fo - Fc | / \sum_{hkl} | Fo |$, where Fc is the calculated and Fo is the observed structure factor amplitude of reflection hkl for the working / free (5%) set, respectively.