Structural insights into marine carbohydrate degradation by GH16 family kappa-carrageenases

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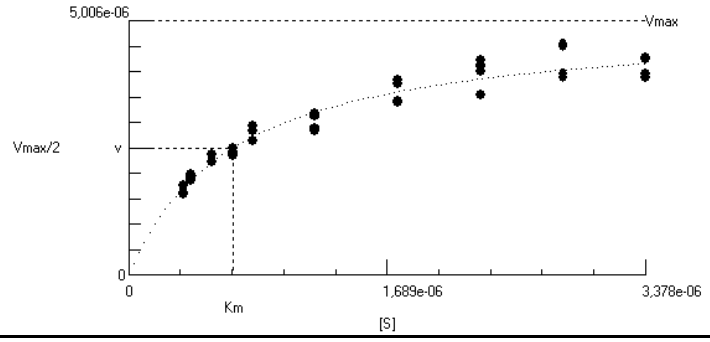
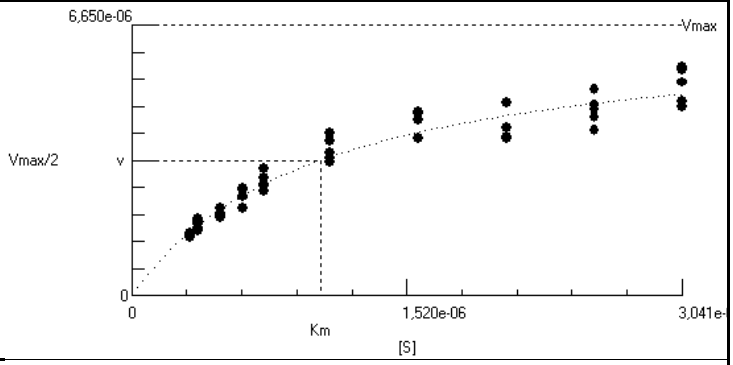
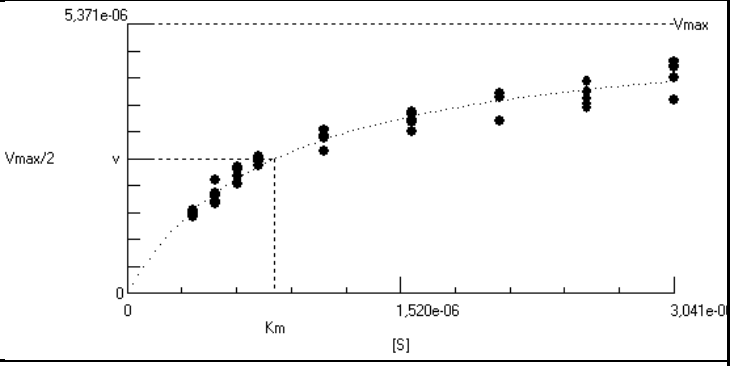
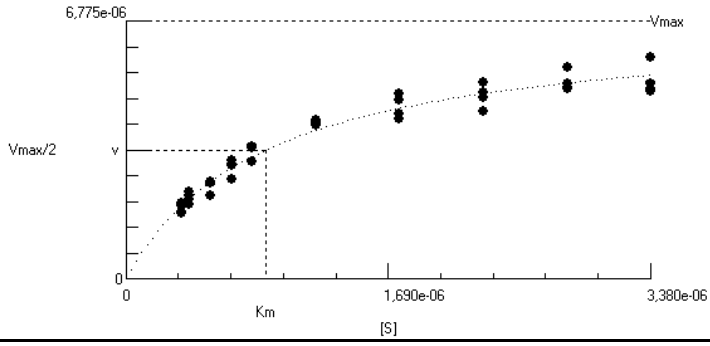
Content:

Table SI: Kinetic parameters and data plot calculated with hyper32 software, with hyperbolic regression model applying weighting

Enzyme name (concentration, nM)	**Vmax** ($M.s^{-1}$)	**Km** (M)	Hyperbolic regression plot
*Pc*CgkA$_{GH16}$ (36.9)	$5.006x10^{-6}$ $\pm 2.409x10^{-7}$	$6.830x10^{-7}$ $\pm 7.486x10^{-8}$	
*Zg*CgkA$_{GH16-CBM16}$ (10.1)	$6.650x10^{-6}$ $\pm 4.665x10^{-7}$	$1.041x10^{-6}$ $\pm 1.343x10^{-7}$	
*Zg*CgkA$_{GH16}$ (5.87)	$5.371x10^{-6}$ $\pm 2.683x10^{-7}$	$8.218x10^{-7}$ $\pm 8.741x10^{-8}$	
*Pc*CgkA$_{GH16-R92A}$ (37.0)	$6.775x10^{-6}$ $\pm 3.784x10^{-7}$	$8.992x10^{-7}$ $\pm 1.011x10^{-7}$	

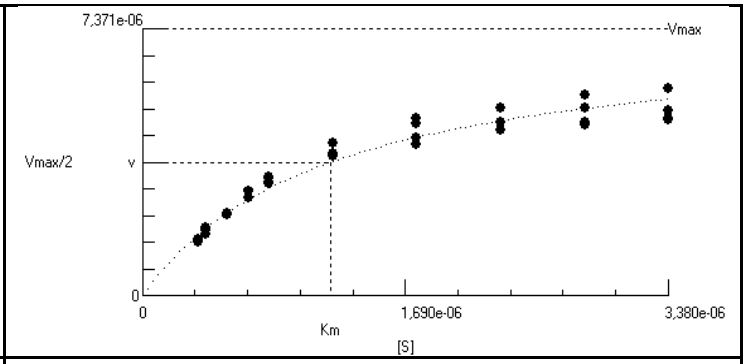
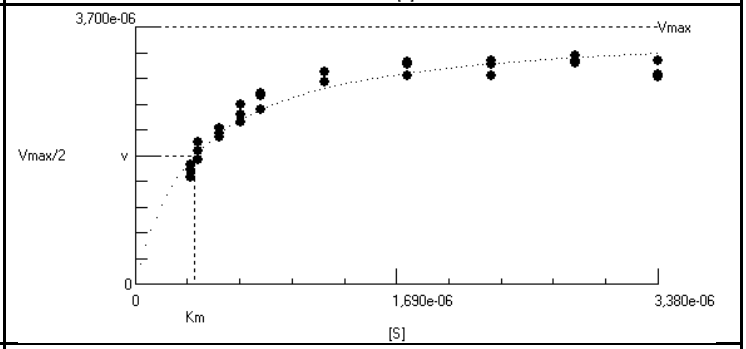
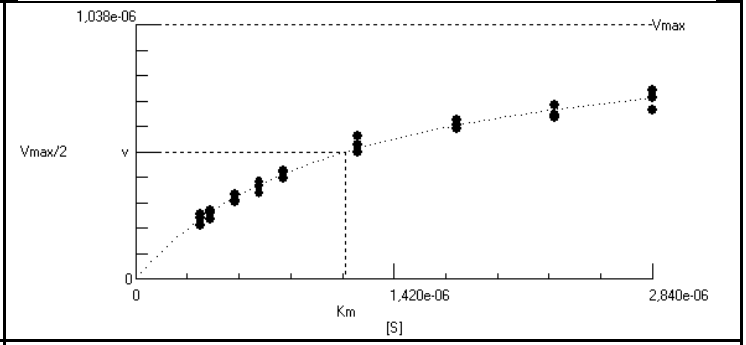
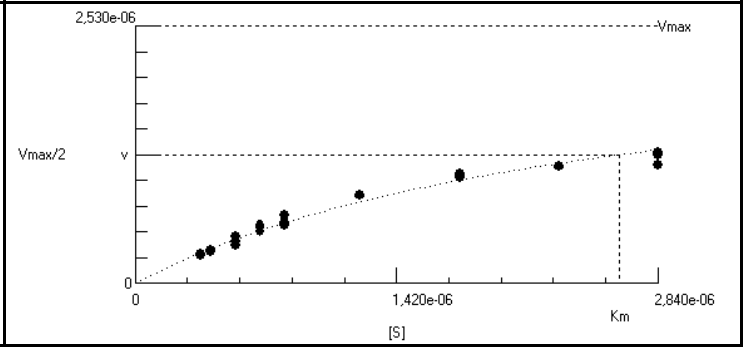
*Pc*CgkA$_{GH16\text{-}R151A}$ (15.4)	$7.371 \times 10^{-6} \pm 4.932 \times 10^{-7}$	$1.207 \times 10^{-6} \pm 1.468 \times 10^{-7}$	
*Pc*CgkA$_{GH16\text{-}Q171A}$ (30.8)	$3.700 \times 10^{-6} \pm 1.846 \times 10^{-7}$	$3.847 \times 10^{-7} \pm 5.877 \times 10^{-8}$	
*Pc*CgkA$_{GH16\text{-}R196A}$ (169.3)	$1.038 \times 10^{-6} \pm 7.204 \times 10^{-8}$	$1.154 \times 10^{-6} \pm 1.404 \times 10^{-7}$	
*Pc*CgkA$_{GH16\text{-}W266A}$ (92.5)	$2.530 \times 10^{-6} \pm 3.480 \times 10^{-7}$	$2.632 \times 10^{-6} \pm 4.964 \times 10^{-7}$	

TABLE SII. Primers sequences used for the cloning of the different κ-carrageenases in the study. Mutated codon are underlined.

	Forward	Reverse
Pc **CgkA$_{GH16-E168D}$**	5' GAAATAGATGTAGTTATTCTACAACAATTCGATG 3'	5' CATCGAATTGTTGTAGAATAACTACATCTATTTC 3'
Pc **CgkA$_{GH16-R92A}$**	5' CTAAGCGAGAATCTCATCAAGCTACATTCTGGGATGGCTGT 3'	5' ACAGCCATCCCAGAATGTAGCTTGATGAGATTCTCGCTTAG 3'
Pc **CgkA$_{GH16-R151A}$**	5' TGGATGTATAGCACCATTGATGCATCATTAACGAAAGAAGGGGA 3'	5' TCCCCTTCTTTCGTTAATGATGCATCAATGGTGCTATACATCCA 3'
Pc **CgkA$_{GH16-Q171A}$**	5' GCGAAATAGACGTAGTGGAGTTAACTGCAAAAAGTGCAGTGAGAGAGT 3'	5' ACTCTCTCACTGCACTTTTTGCAGTTAACTCCACTACGTCTATTTCGC 3'
Pc **CgkA$_{GH16-R196A}$**	5' ATGGAAAACCAACATGGATGGCGCCAGGGTCTTTTCCGCAG 3'	5' CTGCGGAAAAGACCCTGGCGCCATCCATGTTGGTTTTCCAT 3'
Pc **CgkA$_{GH16-R260A}$**	5' CTCACATTATCACAAGGCTTAGCGGCGCCGCATACACAATGG 3'	5' CCATTGTGTATGCGGCGCCGCTAAGCCTTGTGATAATGTGAG 3'
Pc **CgkA$_{GH16-W266A}$**	5' GCGCGCCGCATACACAAGCGAAATGTAATCAATTTTACCCA 3'	5' TGGGTAAAATTGATTACATTTCGCTTGTGTATGCGGCGCGC 3'
Zg **CgkA$_{GH16}$**	5' AAAAAAGGATCCCAACAACCTACGAAGACTTCAAATC 3'	5' TTTTTTCTGCAGTTATGATTTTTCCCAGACCCGAACGTA 3'
Zg **CgkA$_{GH16-CBM16-PorSS}$**	5' AAAAAAGGATCCCAACAACCTACGAAGACTTCAAATC 3'	5' TTTTTTCTGCAGTTACTCCACGAGTATCTTTTTTGAAAC 3'

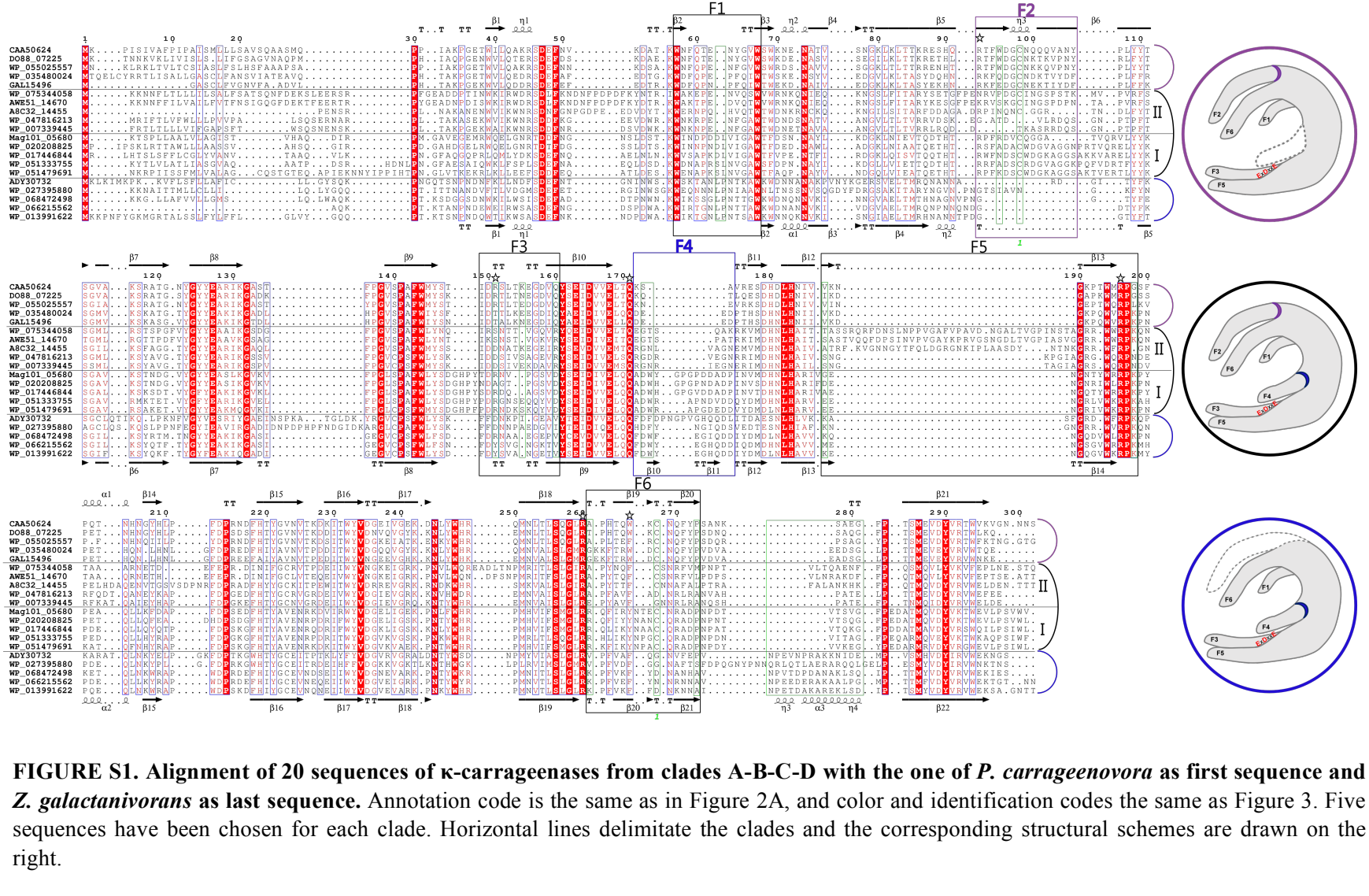


FIGURE S1. Alignment of 20 sequences of κ-carrageenases from clades A-B-C-D with the one of *P. carrageenovora* as first sequence and *Z. galactanivorans* as last sequence. Annotation code is the same as in Figure 2A, and color and identification codes the same as Figure 3. Five sequences have been chosen for each clade. Horizontal lines delimitate the clades and the corresponding structural schemes are drawn on the right.

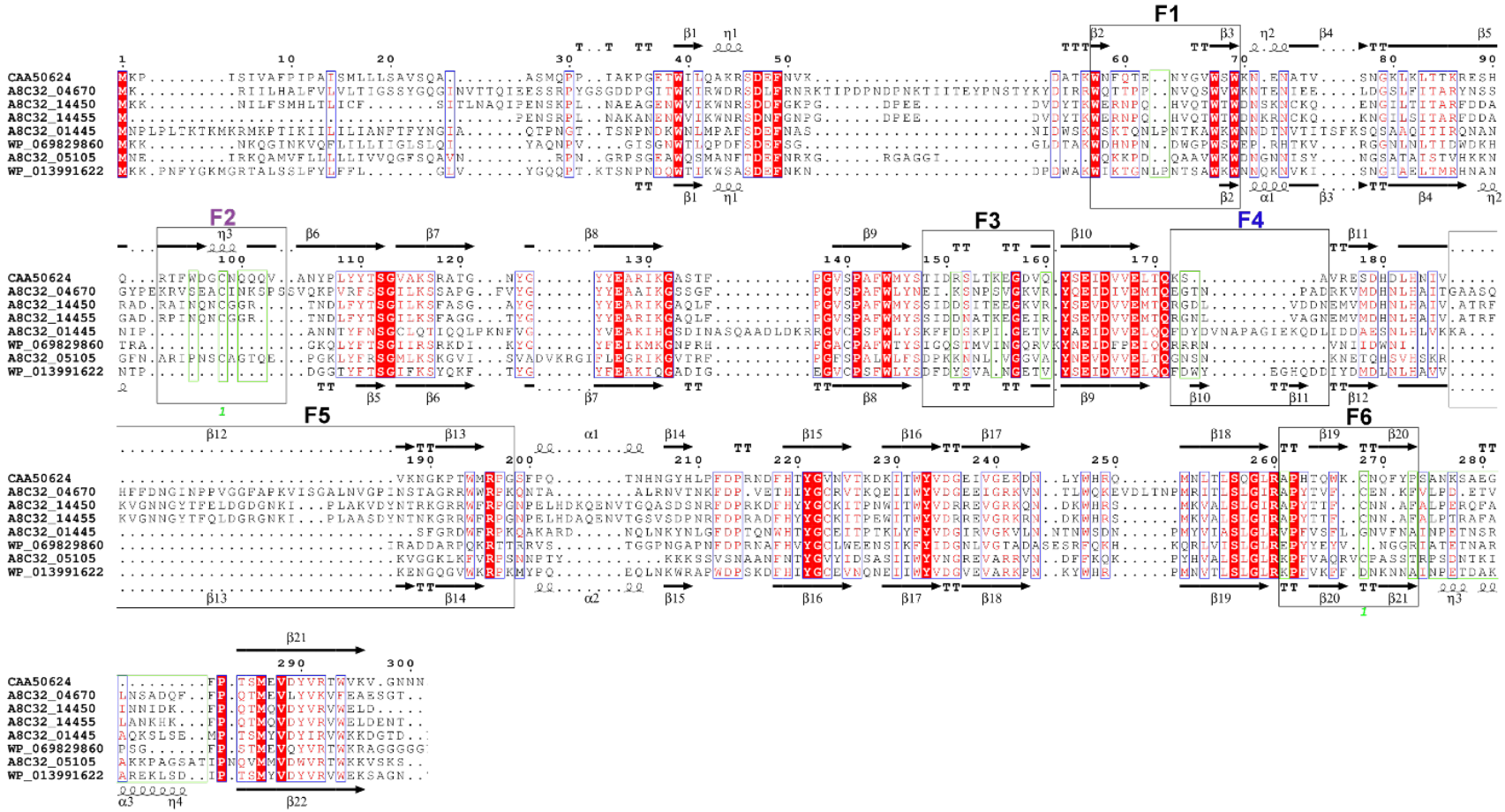


FIGURE S2. Alignment of the six κ-carrageenases of *Algibacter* sp. SK-16 with those of *P. carrageenovora* (first sequence) and *Z. galactanivorans* (last sequence). Annotation code is the same as in Figure 2A.

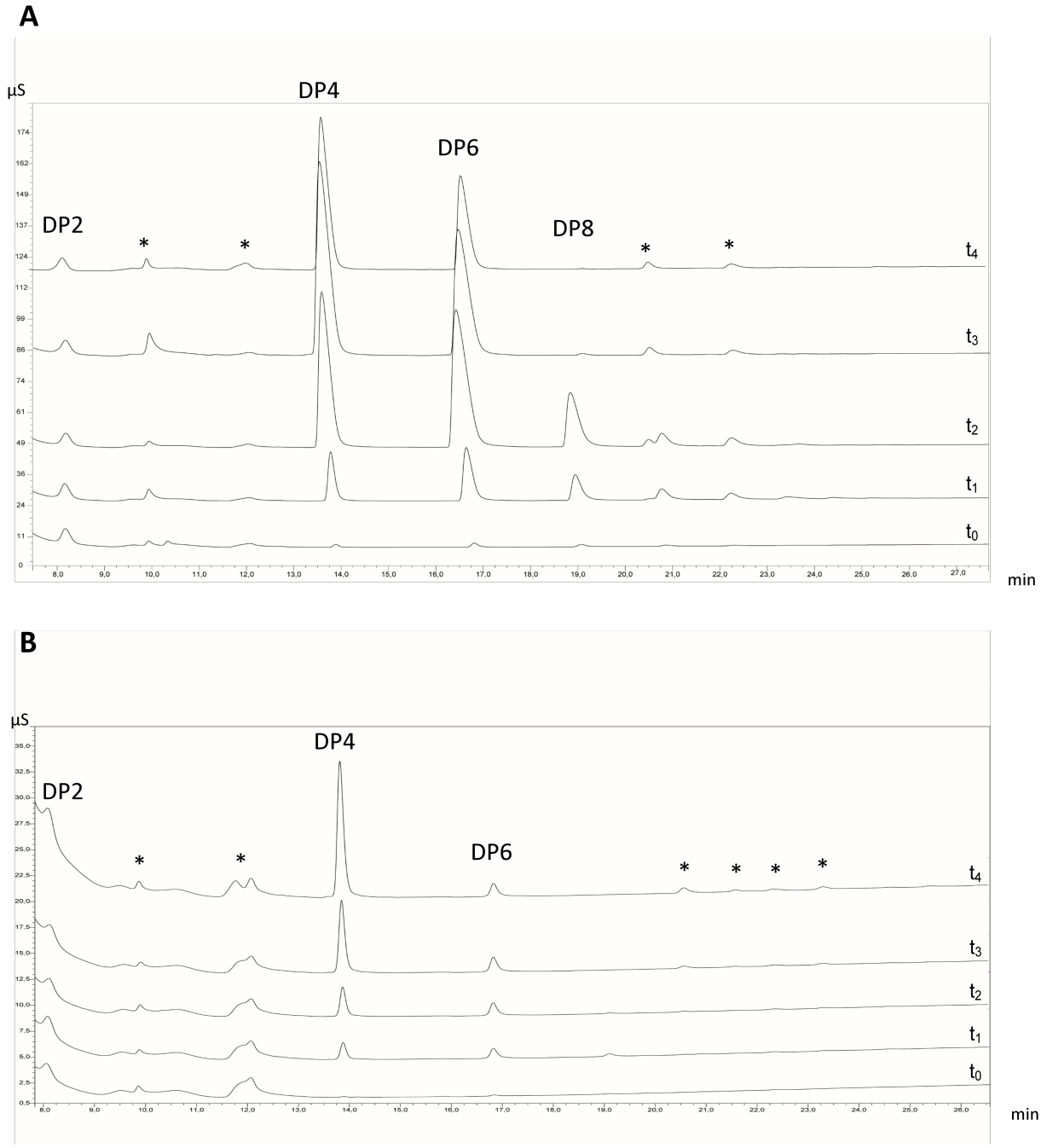


FIGURE S3 Examples of chromatograms obtained for the degradation products of *Zg*CgkA$_{GH16}$ in solution (A) and *Zg*CgkA$_{GH16\text{-}CBM16\text{-}PorSS}$ in microgel (B). In solution we can notice the presence of a peak corresponding to DP2 the height of which remains constant throughout the kinetic experiment. Conversely, in microgel DP2 co-elutes with the salt peak of KCl obstructing a clear interpretation. Asterisks symbolize uncharacterized contaminant peak. Curves correspond to kinetics at t_0=0, t_1=15 min, t_2=45 min, t_3=24 h, t_4=1 week

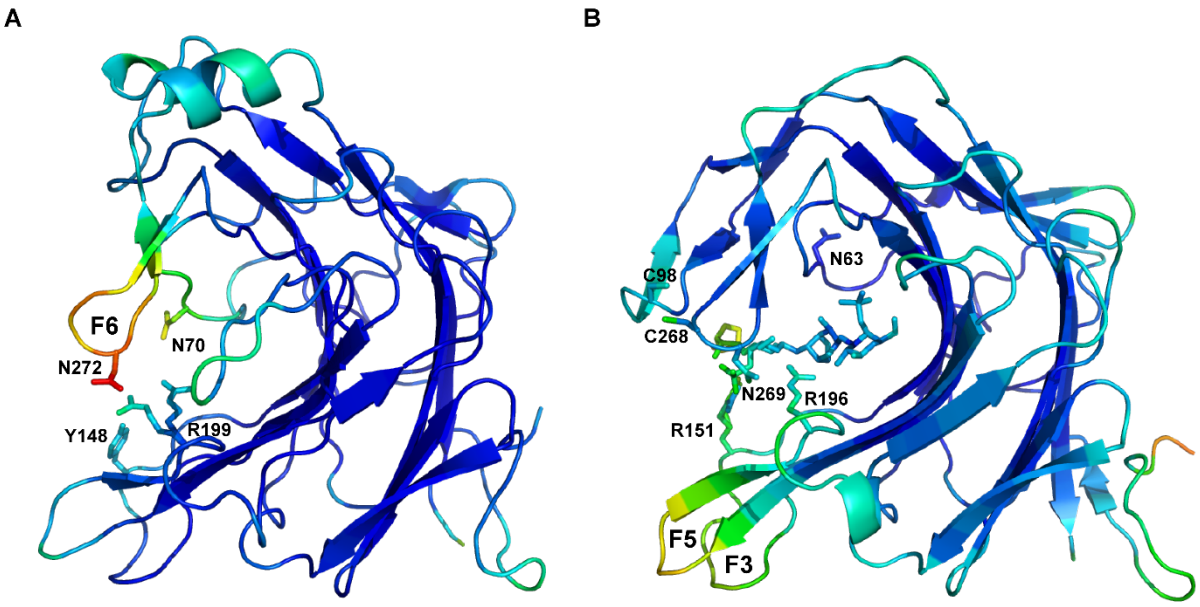


FIGURE S4. Structures of *Zg*CgkA$_{GH16}$ (A) and *Pc*CgkA$_{GH16\text{-}E168D}$ in complex with a κ-neocarratetraose (B) tinted by B factor intensity. Key residues are annotated, as well as the loops with the highest B-factors.

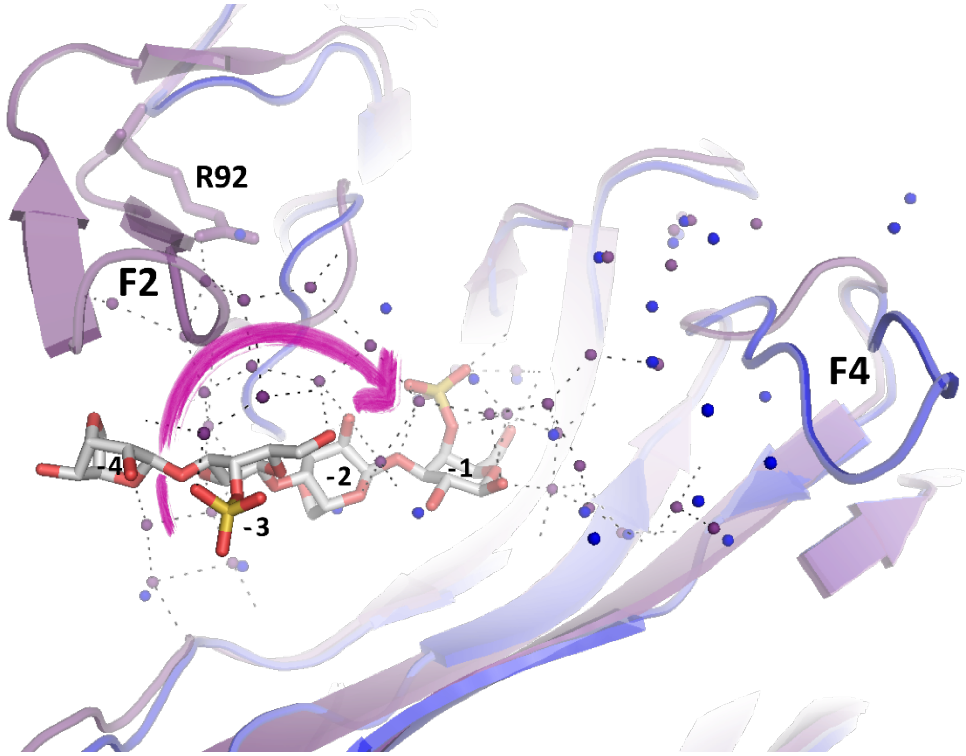


FIGURE S5. Water mesh within the catalytic tunnel of *Pc*CgkA$_{GH16-E168D}$ (cartoon in slate and water in purple) superposed with *Zg*CgkA$_{GH16}$ and its associated water molecules (in blue and marine blue respectively). Possible hydrogen bonding are represented as black dashes. The R92 is shown for its role in the structuration of the water mesh at the entrance of the channel. Purple arrow underline the roll shape of the water mesh around the substrate. Neocarratetraose (DA-G4S-DA-G4S) is colored in light grey and the moieties are labelled with their corresponding positioning in the catalytic subsites from -4 to -1

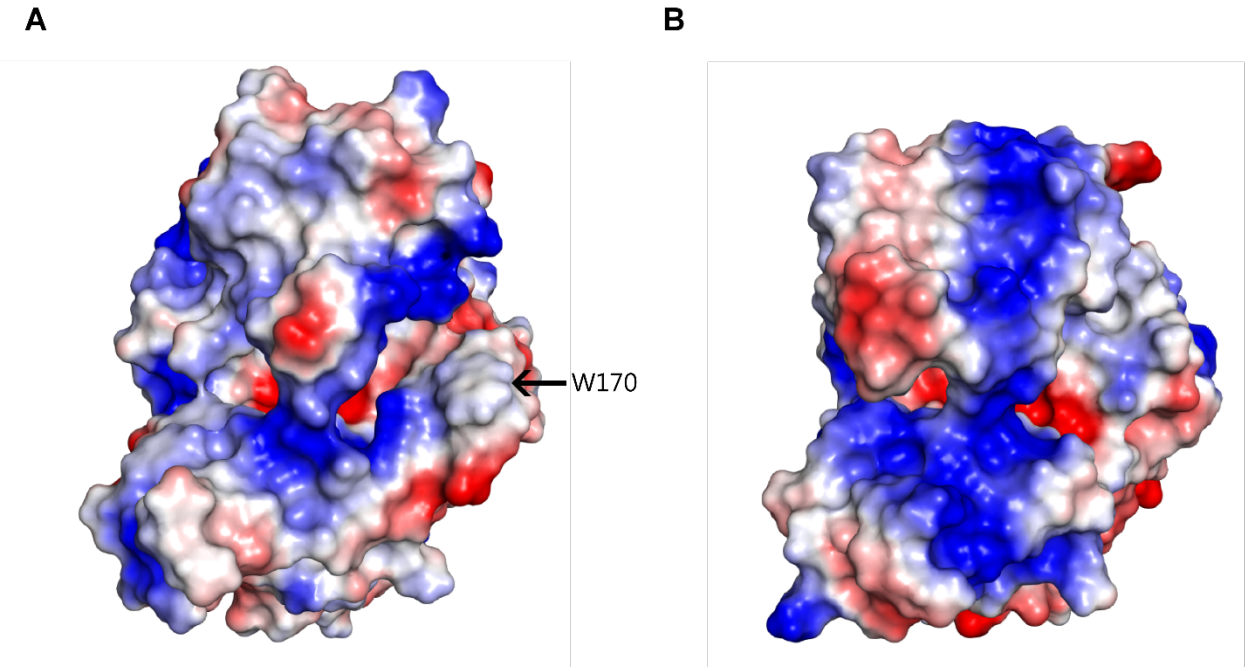


FIGURE S6. Electrostatic map of *Zg*CgkA$_{GH16}$ (A) and *Pc*CgkA$_{GH16\text{-}E168D}$ (B). Color code spreads from blue to red for basic to acidic residues. The position of W170 in *Zg*CgkA$_{GH16}$ is indicated by an arrow.