Supplementary Figures and Table to

Structural analysis of the endogenous glycoallergen Hev b 2 (endo-β-1,3-glucanase) from *Hevea brasiliensis* and its recognition by human basophils

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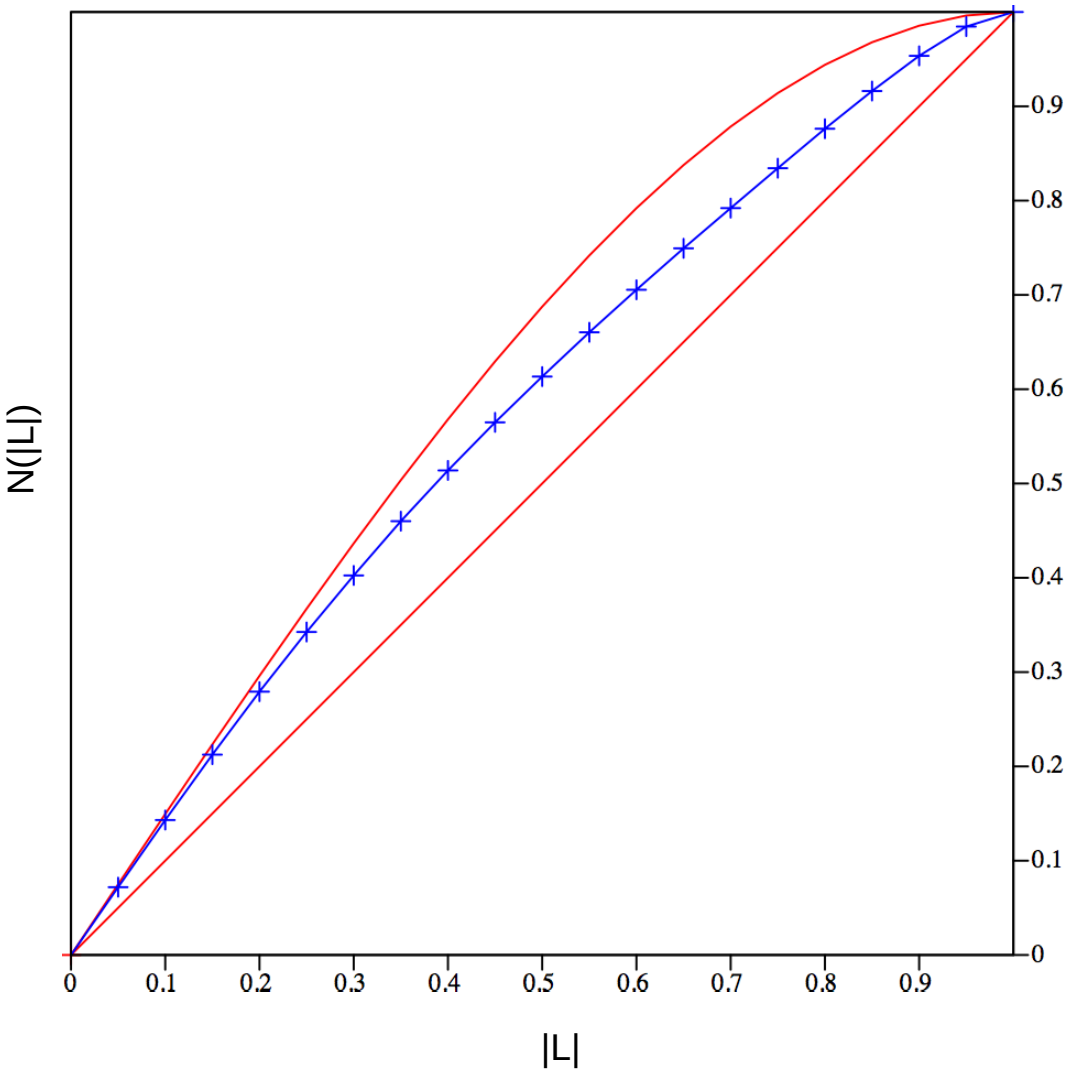
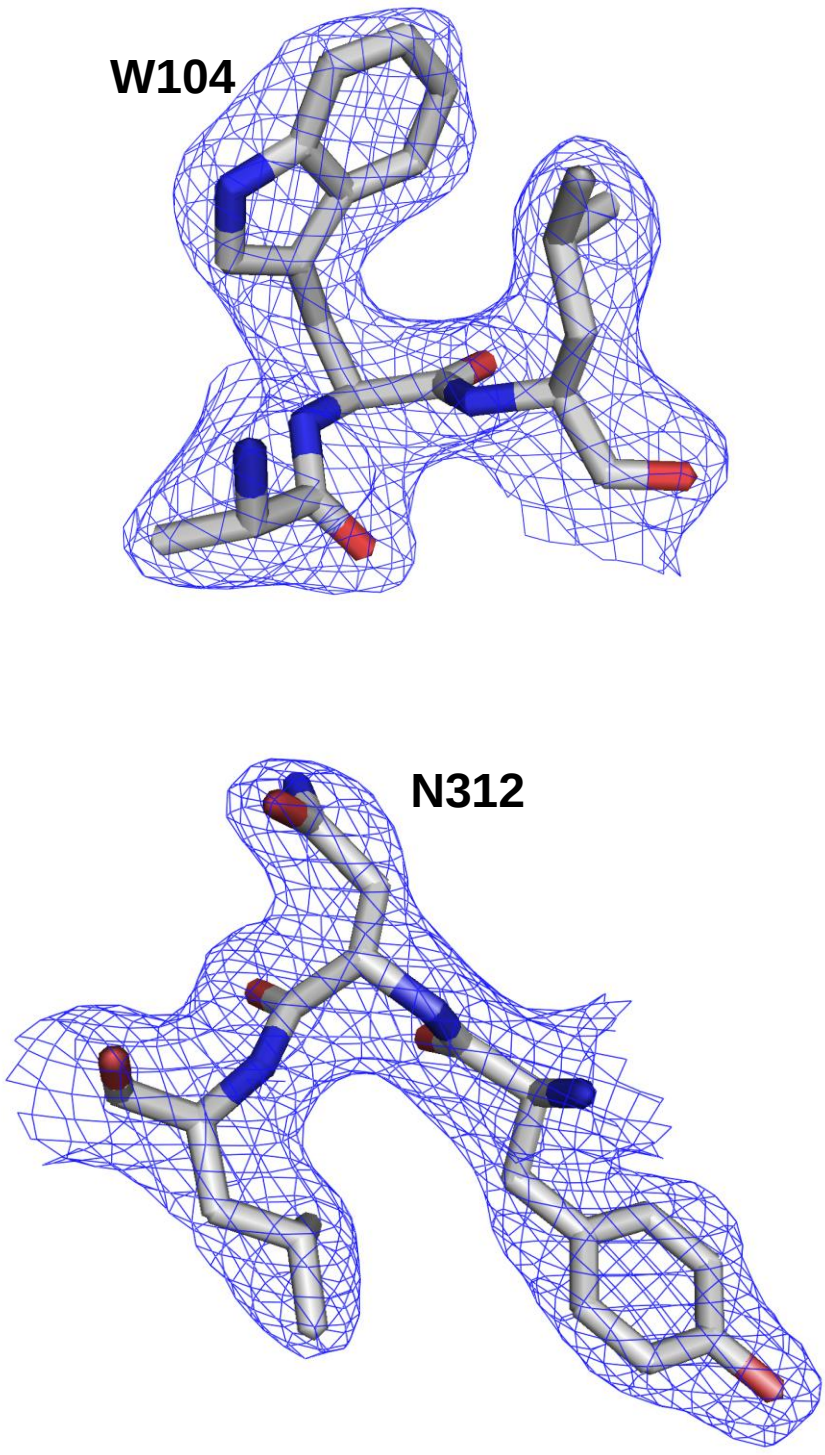
Supplementary Table S1. Proposed structures for the N-glycans in the isoform II of Hev b 2. Glycans were released from the protein by digestion with PNGaseF or PNGaseA. Glycans were labelled with 2-amido benzamide and separated by HPLC. Relative contents were calculated after normalizing with a maltose internal standard. N: N-Acetyl glucosamine, F: Fucose. M: Mannose. X: Xylose. Monosaccharides are listed starting from their non-reducing end. Unless otherwise noted, all glycans were obtained after digestion with PNGase F and Fuc are in a (□1,6) linkage.

Glucose units[a]	Relative content, %	Proposed structure
1.94	-	Maltose, internal standard
2.76	1.30	MN2
2.93	0.42	MN2F
3.03	0.40	MN2F
4.70	0.24	M3N2 or M4N2
5.15	2.84	M3FN2
5.63	0.56	N2M3N2
6.07	0.23	M5N2
6.33	0.04	M5N2
6.55	0.19	M5N2 or M6N2
6.6[b]	92.5	M3XN2F (α1,3)
7.18	1.10	M6N2
8.07	0.05	M7N2
8.46	0.16	M8N2

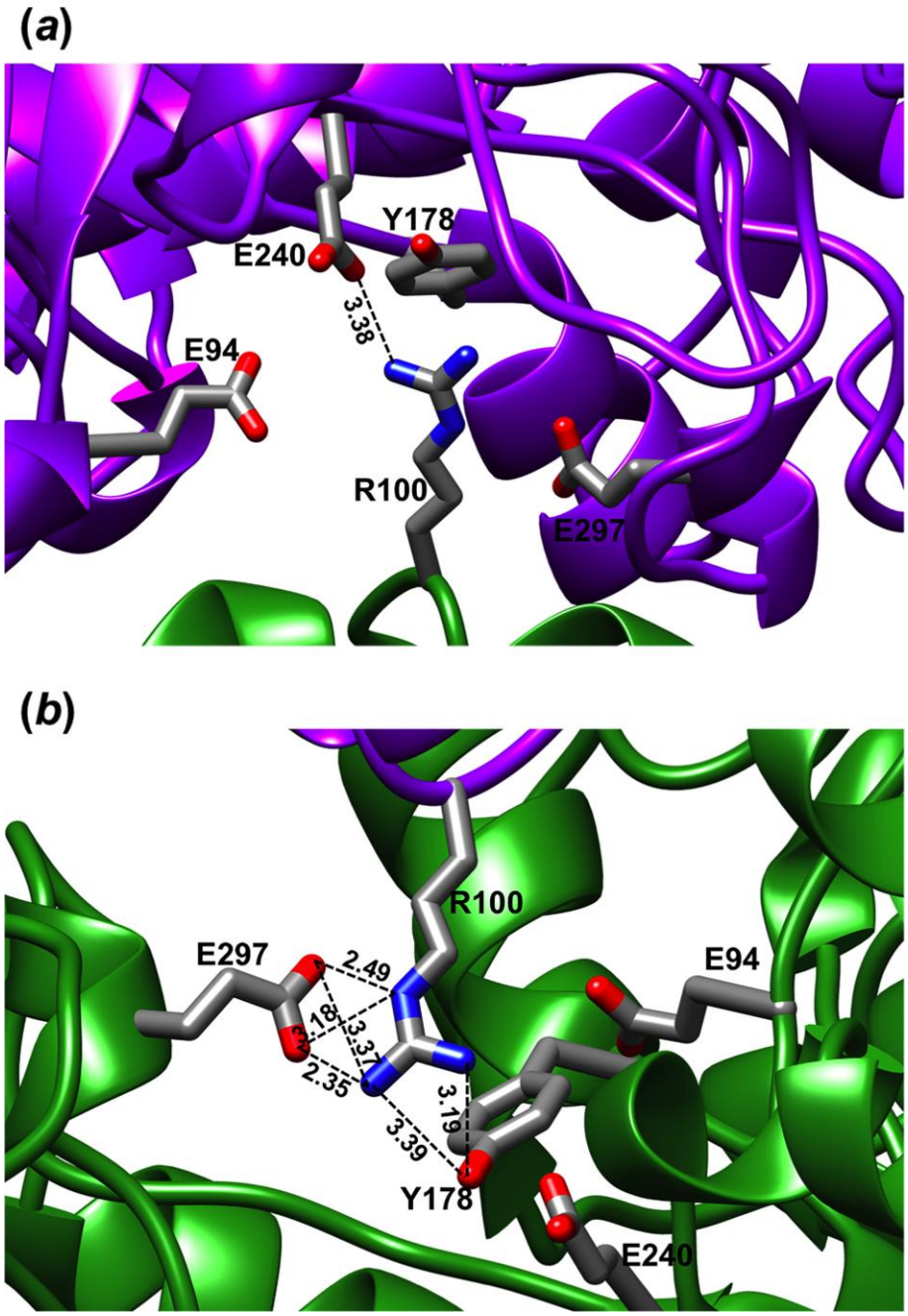
a. As determined with a glucose homopolymer standard.
b. Glycan obtained after digestion with PNGase A.



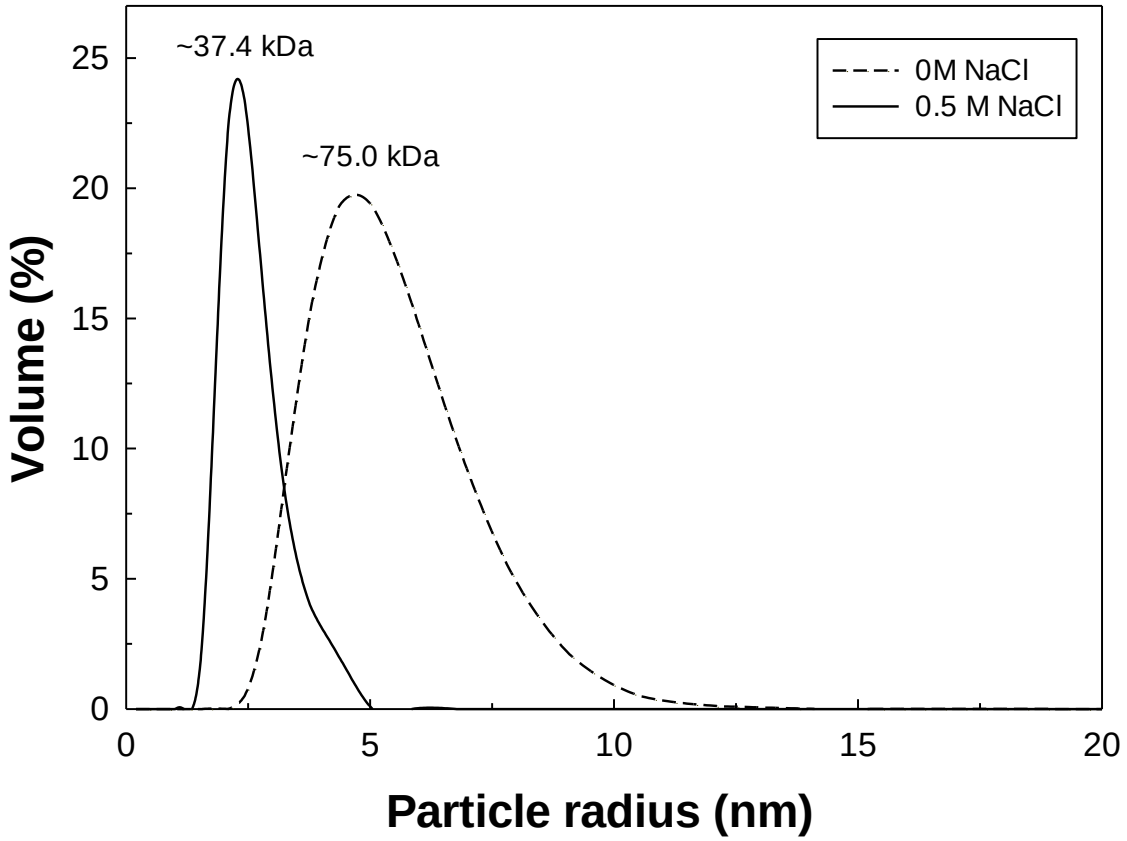
Supplementary Fig. S1. Analysis of crystal twinning for the *P*41 polymorph. DATAMAN Local Intensity Statistics plot (Padilla & Yeates, 2003) showing the Cumulative N(|L|) vs. |L| (acentrics). L is defined as $[I(h_1) - I(h_2)]/[I(h_1) + I(h_2)]$ for unrelated reflections h_1 and h_2. N(|L|) is the theoretical cumulative distribution of |L|, valid for $0 \leq |L| \leq 1$. The blue line represents the experimental data. The upper red line corresponds to expected twin data, while the lower red one represents expected untwinned data.



Supplementary Fig. S2. 2*F*o-*F*c electron density maps contoured at 1.0 σ of the two residues (W104 and N312) that lie outside the expected regions in the Ramachandran plot. They are located in the substrate/product binding groove.



Supplementary Fig. S3. Ribbon representation of the Hev b 2 (**A**-**D**) dimer interface in the *P*41 assembly. Several salt bridges and hydrogen bonds (dashed lines) are observed and distances are indicated. a) Arg100**A** (green) and its interactions with **D** (purple). b) Arg100**D** and its interactions with **A**. A short contact is observed between Nω′H2-Arg100**D** and Oε1-Glu297**A** (2.35 A□).



Supplementary Fig. S4. Dynamic light scattering analysis of Hev b 2 (isoform II glucanase). These data were obtained using a NanoZetasizer-HT dynamic light-scattering (DLS) device from MALVERN Instruments at 20 °C and fitted using the DTS software (Malvern Instruments, Malvern, Worcestershire, UK). The filtered protein samples (pore size, 0.22 μm) were placed in a quartz cuvette (300 μL) and used to verify that at a concentration higher that 0.15 mg ml^{-1}, in sodium citrate buffer pH 5.0, the protein dimerizes. When NaCl (0.5M) is added to the protein the monomeric form is detected. At least 10 measurements were collected for each sample. Size distributions in percentage volume were calculated using MALVERN Instruments software by approximating the protein as a spherical object. The graph shows a superposition of both experiments with a distribution of particle sizes in kDa.