Supplemental data

Functional and structural characterization of an α-(1→2) branching sucrase derived from DSR-E glucansucrase*

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*Running title: *3D structure of an α-(1→2) branching sucrase*

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Supplemental data for the Experimental Procedure section

TABLE S1: Primers used to generate GBD-CD2 truncated mutants.
Twelve truncation sites were chosen. Green colored bases were necessary for the directional insertion of the purified PCR products in pBAD TOPO Directional 102 vector. Base numbering corresponds to base annotation of the *dsr-E* gene sequence as deposited in the GenBank (accession number AJ430204.1). Underlined bases correspond to the NcoI restriction site; red colored bases have been mutated to create the NcoI restriction site.

Primer name	Sequence (5' to 3')
For_pBadD_ΔN_{180}	CACCATGG$_{4274}$ATAGTCAAAATCAATGGCATTATTTCT
For_pBadD_ΔN_{173}	CACCATGG$_{4487}$CAGATAAGTCTTGGCTTTACCCTAATG
For_pBadD_ΔN_{166}	CACCATGGC$_{4682}$AGATGGCTCATGGTTATATTTGAATG
For_pBadD_ΔN_{161}	CACCATGGT$_{4806}$ATCCGATGGTTCATGGGTGTATTTGG
For_pBadD_ΔN_{154}	CACCATGGC$_{5003}$AGATGGTTCATGGTTATATCTAGAAG
For_pBadD_ΔN_{147}	CACCATGG$_{5195}$GCGATAAAATCATGTATTTTGATGAAG
For_pBadD_ΔN_{145}	CACCATGG$_{5258}$ATGGTTCATGGTATTACTTAAATCAGG
For_pBadD_ΔN_{137}	CACCATGGC$_{5451}$AGATGGTTCATGGATTTATCTTGATG
For_pBadD_ΔN_{130}	CACCATGGC$_{5646}$ACCTGGTGTATGGGCTTACTTTGGTG
For_pBadD_ΔN_{123}	CACCATGGC$_{5838}$ACAAGCAGGTCACTATATCACGAAAA
For_pBadD_ΔN_{115}	CACCATGGA$_{6063}$TTACGGTACAATAACACTCAAGCAAG
For_pBadD_ΔN_{106}	CACCATGG$_{6296}$ATGGTCATTATATTTCCCAAGATGGCC
Rev_pBadD	AGCTTGAGGTAATGTTGATTTATC

Effect of calcium chloride - Hydrolytic and transferase activities of ΔN_{123}-GBD-CD2 were determined in the presence of calcium chloride (3.4 mM), EDTA (1 mM and 5 mM), or without any additive, by measuring the amount of reducing sugars produced from sucrose consumption. Triplicates of initial velocity determinations were carried out at standard conditions, with an enzyme concentration of 0.32 $U.mL^{-1}$ (87 nM). Analogous experiments were done in standard conditions in the presence of 62 mM (concentration expressed in anhydroglucosyl units) of 70 kDa dextran (Sigma-Aldrich) with 0.10 $U.mL^{-1}$ (27 nM) of enzyme.

Mutagenesis studies - Construction of the mutants

Mutagenesis efforts were focused on three non-conserved amino acids: A2249, G2250, and F2214. Inverse PCR reactions were carried using the set of primers listed in table S2 and plasmid pBad-TOPO-D102 (GE Healthcare Biosciences) containing the *ΔN_{123}-gbd-cd2* encoding gene as template. PCR amplification was carried out with Phusion DNA polymerase (0.5 U) for 16 cycles (95 °C, 15 s; 55 or 60°C, 20 s; 72 °C, 8 min). PCR products were digested with DpnI to eliminate the methylated parental template and purified using a Qiaquick spin column, following the manufacturer recommendations. *E. coli* TOP10 cells were transformed with the plasmid and plated on LB agar supplemented with 100 µg/mL ampicillin. For each construction, ten clones were isolated and their corresponding plasmids stored at -20 °C. Restriction analyses using the specific silent restriction sites were performed when possible and three matching mutants of each construction were sequenced on the entire gene by GATC-Biotech AG (Mulhouse, France) and showed no other mutations.
Ampicillin was purchased from Euromedex (Souffelweyersheim, France). DpnI restriction enzyme and Phusion® High-Fidelity DNA Polymerase were purchased from New England Biolabs (Beverly, MA, USA). Oligonucleotides were synthetized by Eurogenetec (Liège, Belgium). DNA extraction (QIASpin) and purification (QIAQuick) columns were purchased from Qiagen (Chatsworth, CA, USA).

TABLE S2: Primers used to generate the mutants at positions 2214, 2249 and 2250.

Primer Name	Nucleotide Sequence*
A2249Wfor	5'- G GTT GAA **TGG** GGC TTA GAC GCA GGT AC<u>C</u> TCA ACG ATT C -3'
A2249Wrev	5'- G AAT CGT TGA <u>G</u>GT ACC TGC GTC TAA GCC **CCA** TTC AAC C -3'
G2250Wfor	5'- GGT TGA AGC T**TG** GTT AGA CGC AGG TAC -3'
G2250Wrev	5'- GTA CCT GCG TCT AAC **CA**A GCT TCA ACC -3'
A2249D-G2250Wfor	5'- CA TTG GTT GAA G**A**T **T**G**G** TTA GAC GCA GGT ACA TCA ACG -3'
A2249D-G2250Wrev	5'- CG TTG ATG TAC CTG CGT C**T**A **A**C**C** AAT CTT CAA CCA ATG -3'
F2214Nfor	5'- GAC GCT GTC GAC **<u>AA</u>**T ATT CAT AAT GAT AC - 3'
F2214Nrev	5'- GT ATC ATT ATG AAT A**<u>TT</u>** GTC GAC AGC GTC -3'

*bold codons indicate the bases which were used to obtain the replacement by the desired amino acids. Underlined nucleotides indicate the mutations performed to insert silent restriction sites.

Isomaltotriose docking in the model of ΔN_{123}-GBD-CD2 glucosyl enzyme intermediate - Using the structure of the amylosucrase glucosyl-enzyme intermediate (PDB entry 1S46) and its superposition with GTF180-ΔN and ΔN_{123}-GBD-CD2, a model of the ΔN_{123}-GBD-CD2 glucosyl-enzyme intermediate was constructed. For isomaltotriose, PubChem database entry CID 439668 (1) was used as a starting conformation. AutoDock Vina (2) was used to dock isomaltotriose. Selection of models was based on the proper orientation of a C2 hydroxyl of isomaltotriose toward the C1 of the glucosyl enzyme intermediate.

Supplemental data for the Results and Discussion section

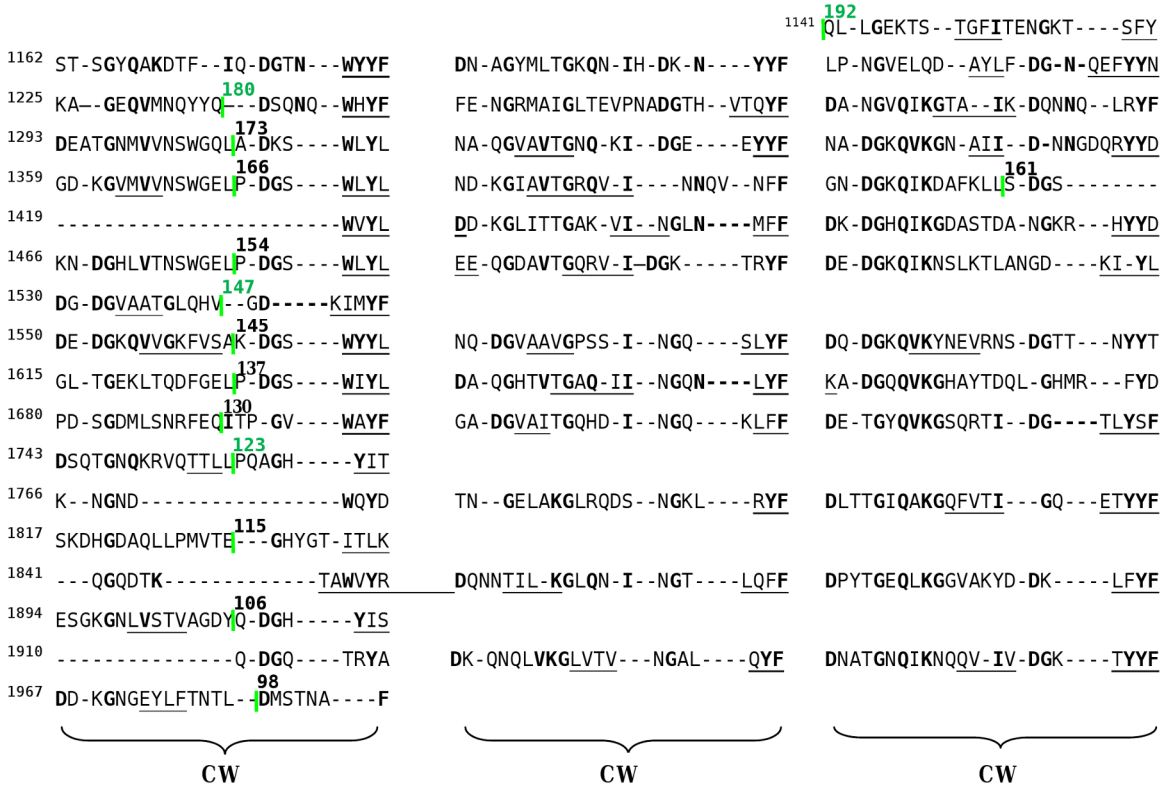

FIGURE S1: Alignment of putative CW-like motifs (Pfam family PF01473) found in the glucan binding domain (GBD) of GBD-CD2 (3). The CW-like motifs of GBD-CD2 were identified based on their similarity with those participating in the solenoid fold of the C-terminal part of autolysine A (C-LytA) of *Streptococcus pneumoniae*. On the left, numbering refers to the DSR-E enzyme sequence. Strongly conserved residues are in bold. Underlined residues represent putative β-strands. Green dashes depict truncation sites within the GBD. Numbers refer to molecular weights in kDa of the different constructs (e.g. 192 kDa corresponds to GBD-CD2, 123 kDa corresponds to the crystallized ΔN_{123}-GBD-CD2 and 98 kDa corresponds to the CD2 catalytic domain). Molecular weights in green refer to expressed and active mutants.

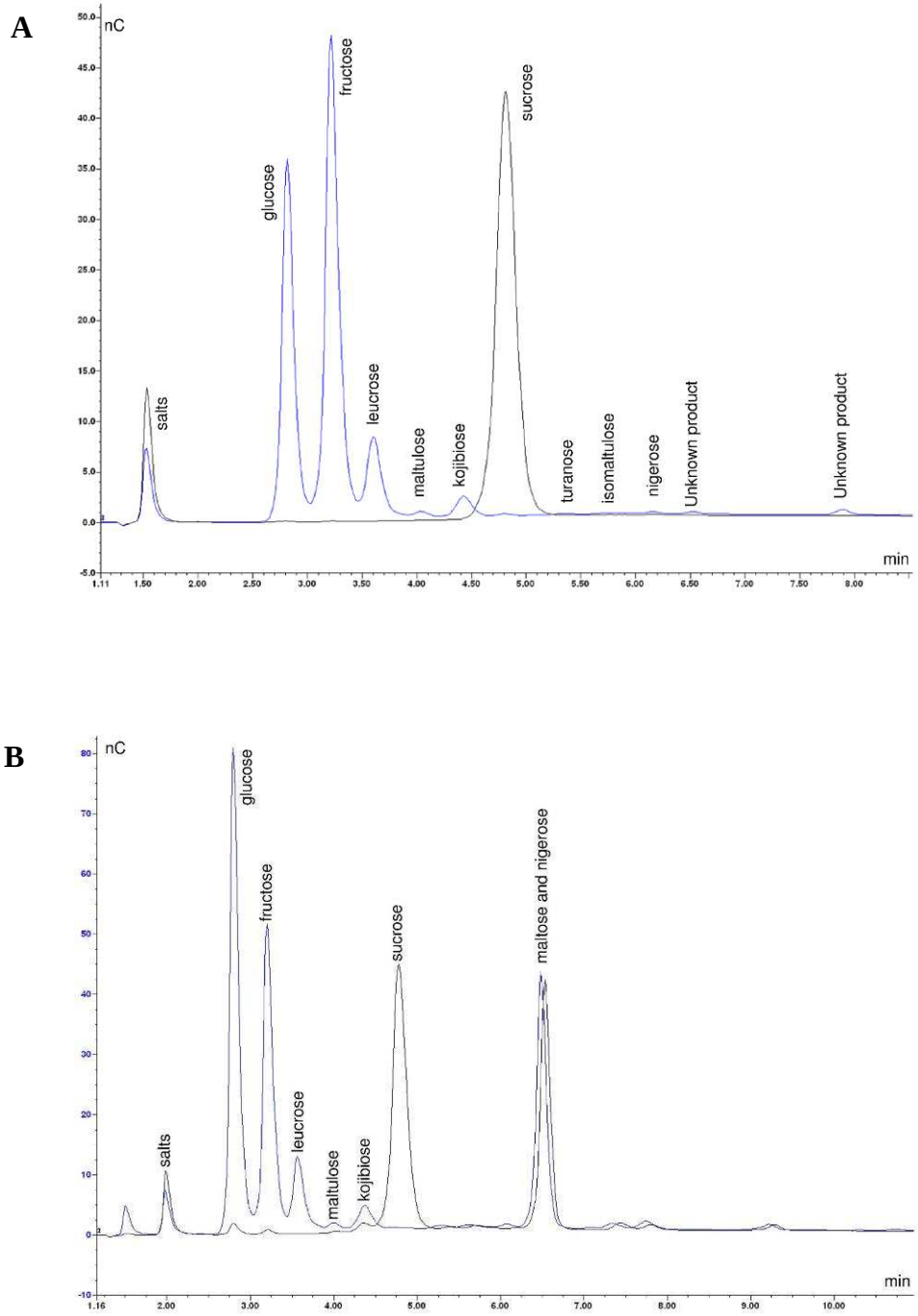

FIGURE S2: Acceptor reactions with ΔN_{123}-GBD-CD2.
High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) chromatograms of the acceptor reactions with ΔN_{123}-GBD-CD2 in the presence of sucrose alone (**A**), or sucrose and maltose (**B**). Black and blue chromatograms depict initial and final time points of the reactions, respectively.

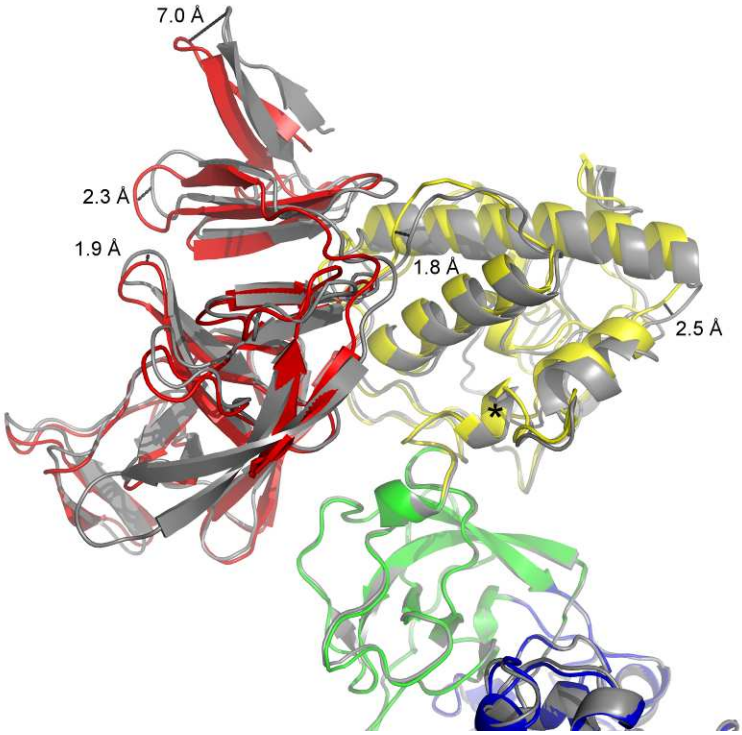

FIGURE S3: Superposition of domains IV and V between ΔN_{123}-GBD-CD2 low and high-resolution structures.
Structural alignment was realized considering domains A, B and C (688 residues out of 1052). Red corresponds to domain V; yellow to domain IV; green to domain B and blue to domain A. Grey or bright colors refer to the structures at 3.3 Å and 1.9 Å resolution, respectively. A star depicts the approximate position of the rotation axis, perpendicular to the plan, about which domains IV and V rotate.

TABLE S3: Effect of calcium chloride and EDTA on ΔN_{123}-GBD-CD2 enzymatic activities. Results are given as means ± standard deviation; n=3. Activities without any of these two compounds were taken as reference (100%).

Compound	Sucrose hydrolysis (%)	Transferase activity (%)
None	100 ± 8	100 ± 2
$CaCl_2$ (3.4 mM)	107 ± 15	113 ± 3
EDTA (1 mM)	88 ± 10	98 ± 4
EDTA (5 mM)	86 ± 9	101 ± 2

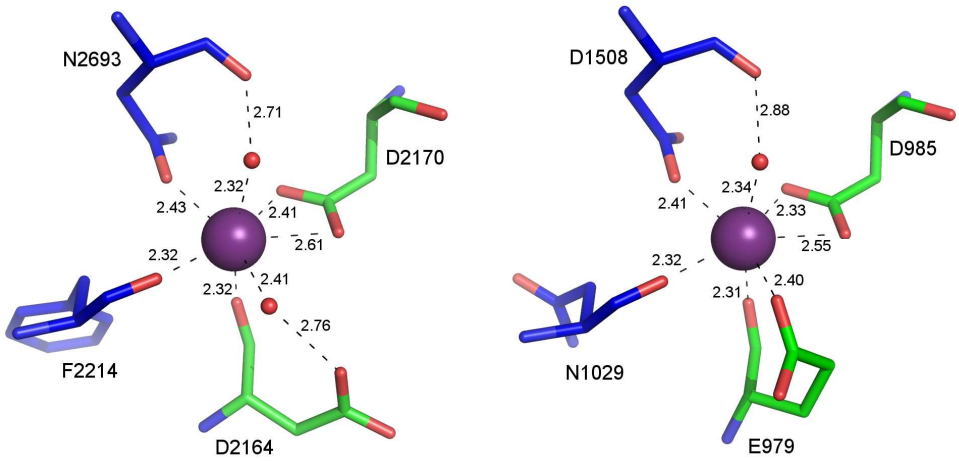

FIGURE S4: Calcium binding sites of ΔN_{123}-GBD-CD2 (left) and GTF180-ΔN (right). Heptacoordinated calcium ions are depicted as purple spheres. Water molecules are shown as red spheres. Distances are indicated in angstrom (4).

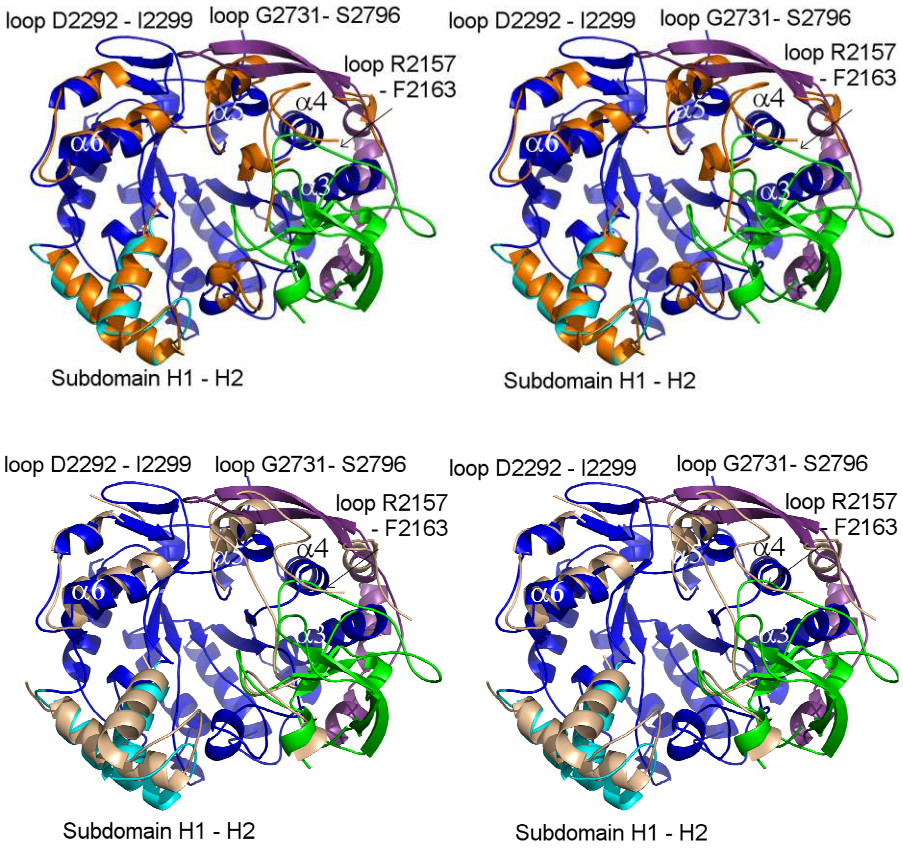

FIGURE S5: Stereo view of secondary structure differences between ΔN_{123}-GBD-CD2 (domain A in blue, cyan and purple; domain B in green), GTF180-ΔN (orange, upper part of the figure), and GTF-SI (wheat, lower part of the figure).

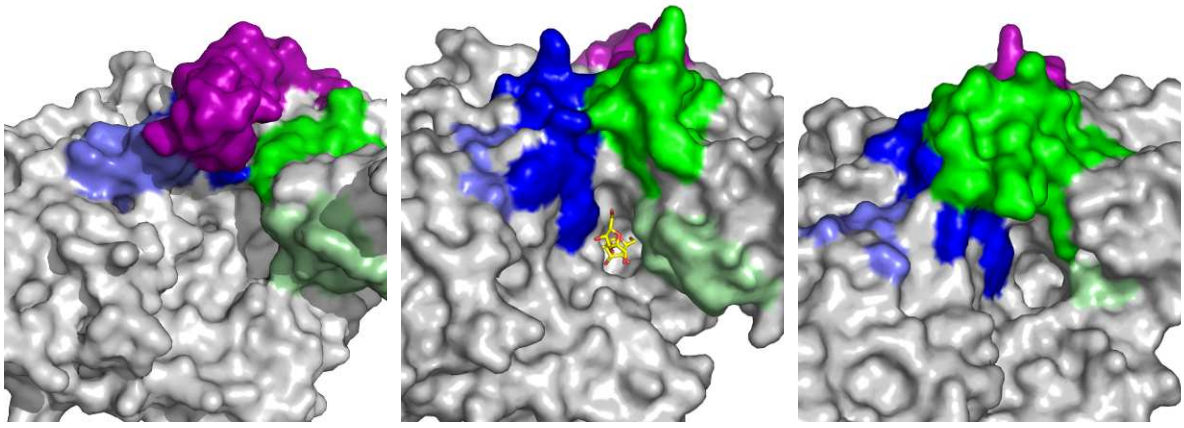

FIGURE S6: Comparison of the loops at the upper part of the catalytic gorges of ΔN_{123}-GBD-CD2 (left), GTF180-ΔN (middle) and GTF-SI (right). The sucrose molecule displayed in subsites -1 and +1 is that of the GTF180-ΔN:sucrose complex structure. For ΔN_{123}-GBD-CD, the loop from G2731 to S2796 is in purple, the loop from D2292 to I2299 in slate blue, helix α_5 from the $(\beta/\alpha)_8$ barrel in blue, the loop from R2157 to F2163 in green, and the loop from H2129 to G2138 in pale green.

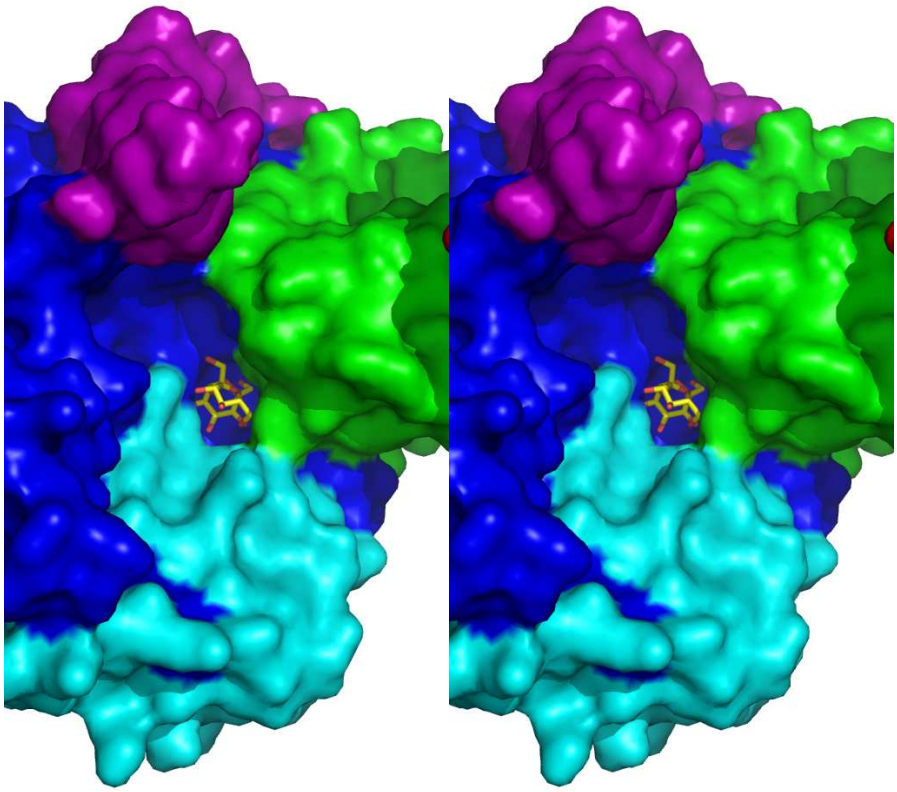

FIGURE S7: Stereo-view of the catalytic site of ΔN_{123}-GBD-CD2. A sucrose molecule (shown in sticks with yellow carbon atoms and oxygen atoms in red) has been introduced in the same position as that found in the structure of the GTF180-ΔN:sucrose complex. Domain A: blue, $(\beta/\alpha)_8$ barrel; cyan, subdomain H1- H2; purple, loop G2731 to S2796 protruding from domain B and contributing to domain A. Domain B: green.

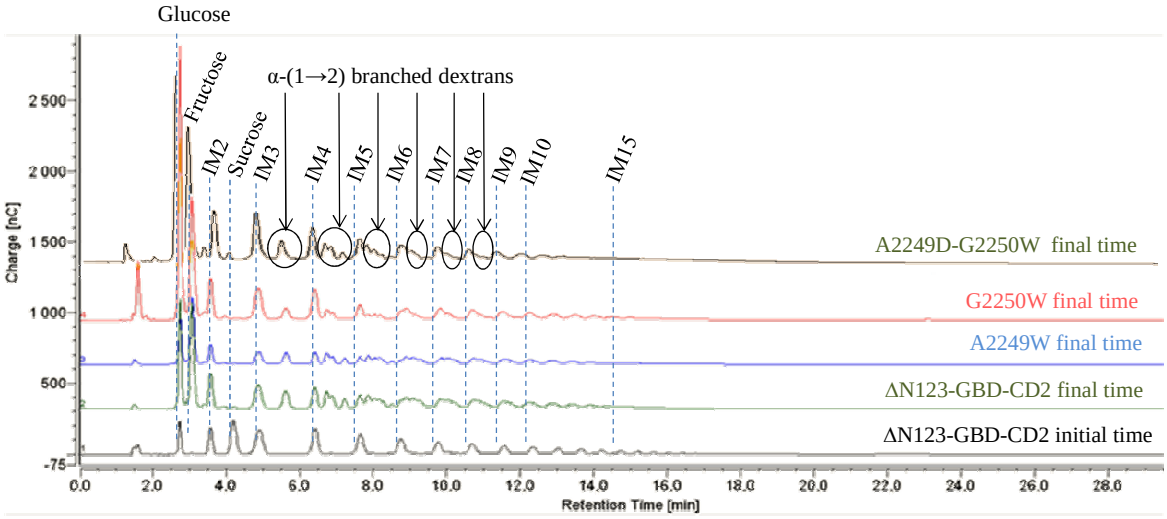

FIGURE S8: HPAEC-PAD chromatograms of 1 kDa dextran acceptor reaction products synthesized with the wild-type enzyme, A2249W, G2250W and AG2249-2250DW mutants.
The products were synthesized from sucrose (146 mM) and 1 kDa dextran (146 mM) using 1 U/mL of enzyme in standard conditions. 1 kDa dextran consists of isomalto-oligosaccharides with polymerization degrees ranging from 2 to 12 (IMn).

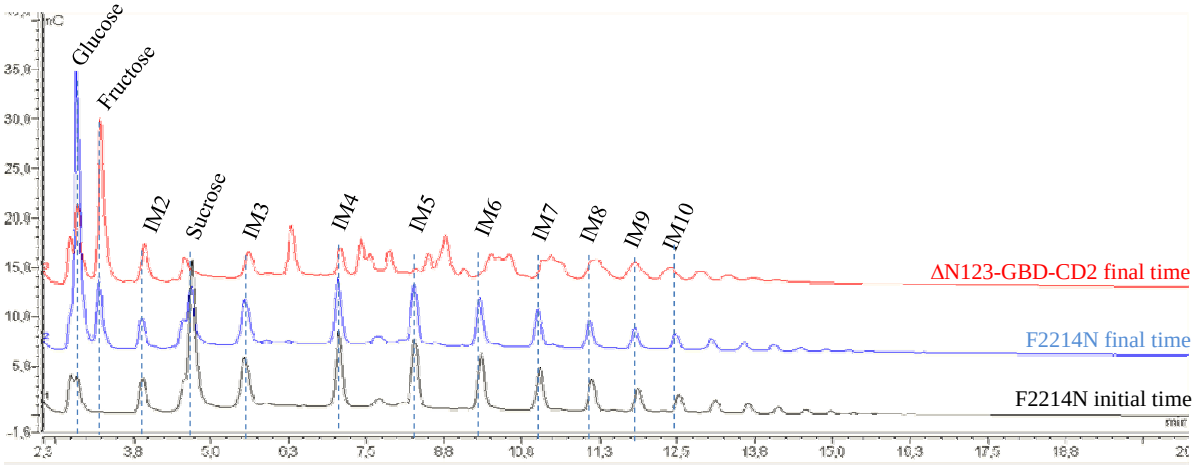

FIGURE S9: HPAEC-PAD chromatograms of 1 kDa dextran acceptor reaction products synthesized with the wild-type enzyme and F2214N mutant.
The products were synthesized from sucrose (146 mM) and 1 kDa dextran (146 mM) using 1 U/mL of enzyme in standard conditions. 1 kDa dextran consists of isomalto-oligosaccharides with polymerization degrees ranging from 2 to 12 (IMn).

References for Supplemental Data

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