Crystallographic snapshot of cellulose synthesis and membrane translocation

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Supplementary Information:

Supplementary Table

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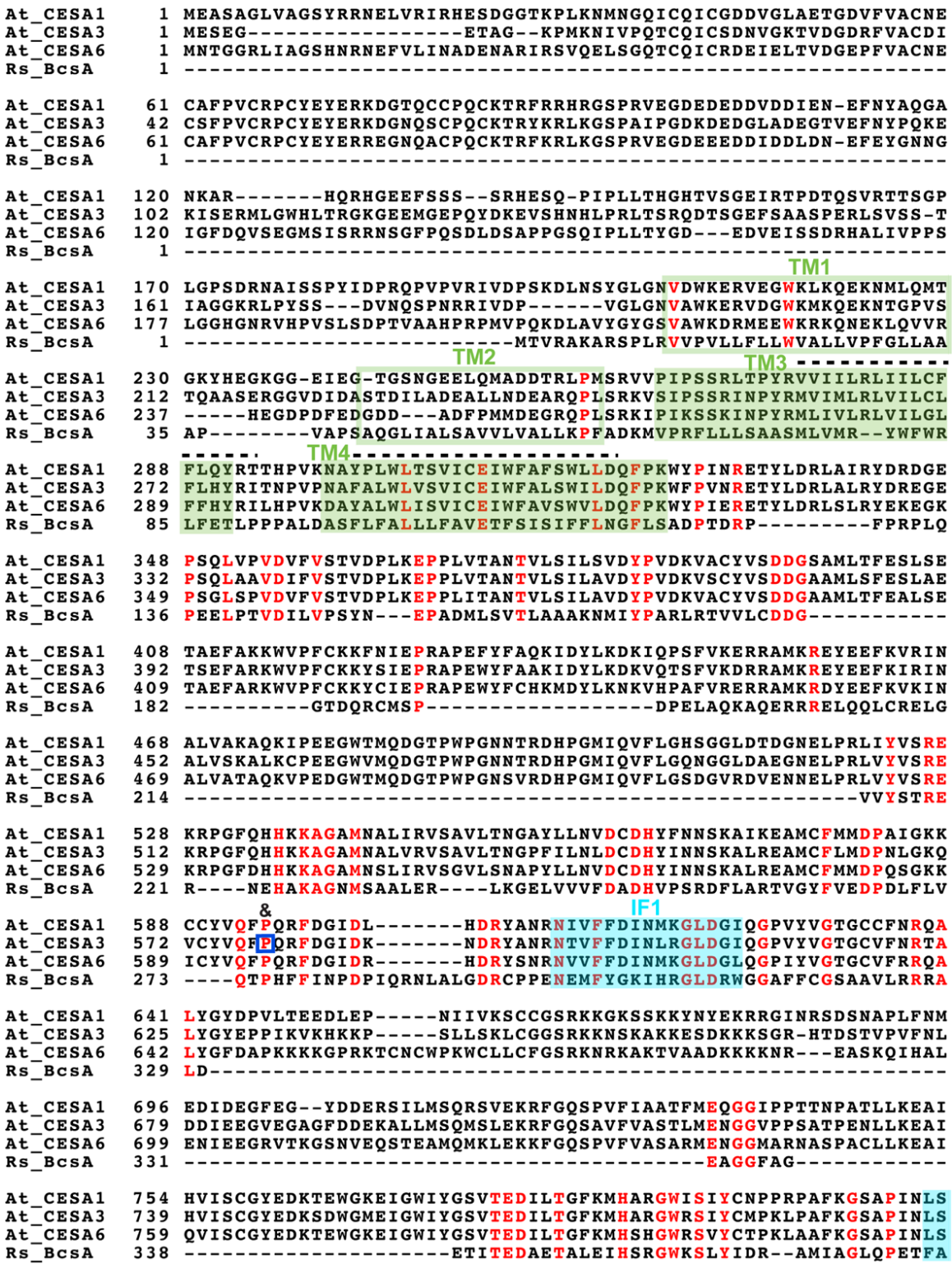
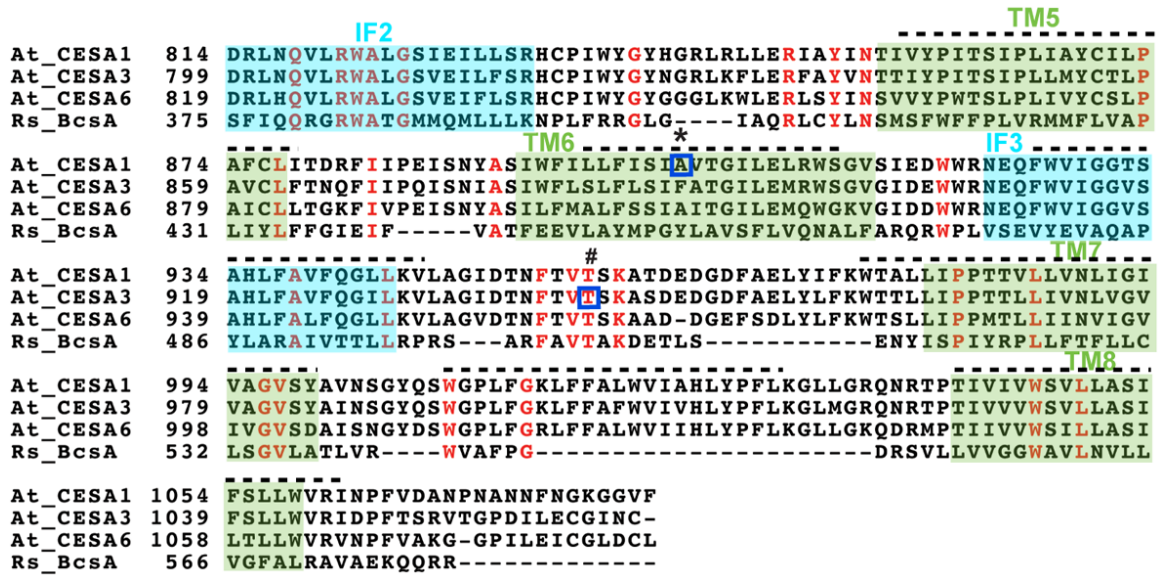
Supplementary Table. Crystallographic data collection and refinement statistics.

	Wild-type	Se-Met-BcsA-B	$SmCl_3$-soaked	EMTS-soaked
Data collection				
Space group	$P4_32_12$	$P4_32_12$	$P4_32_12$	$P4_32_12$
Cell dimensions				
a, *b*, *c* (Å)	103.1, 103.1, 468.3	104.3, 104. 3, 470.5	103.6, 103.6, 470.3	103.0, 103.0, 469.5
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å)	35-3.25 (3.42-3.25) *	50-4.64 (4.89-4.64)	50-5.0 (5.27-5.0)	50-3.97 (4.19-3.97)
Wavelength (Å)	0.97949	0.97949	1.84527	1.00595
R_{meas}	0.1 (0.54)	0.157 (>1.0)	0.18 (0.96)	0.089 (0.28)
Mean I / σI	17 (4.8)	15.1 (3.1)	15.9 (4.2)	29.6 (13.4)
Completeness (%)	99.9 (100.0)	97.9 (100.0)	100.0 (100.0)	99.9 (100.0)
Redundancy	15.2 (15.1)	22.4 (21.5)	26.9 (27.8)	26.8 (27.6)
Refinement				
Resolution (Å)	35-3.25			
No. reflections				
Total	39,037			
R_{free}	1,951			
R_{work} / R_{free}	21.28 / 28.17			
No. atoms				
Protein	10,788			
β-1,4 glucan	199			
UDP	25			
LDAO	25			
B-factors				
Chain A	135.8			
Chain B	148.0			
β-1,4 glucan	119.0			
UDP	89.0			
R.m.s deviations				
Bond lengths (Å)	0.007			
Bond angles (°)	1.37			

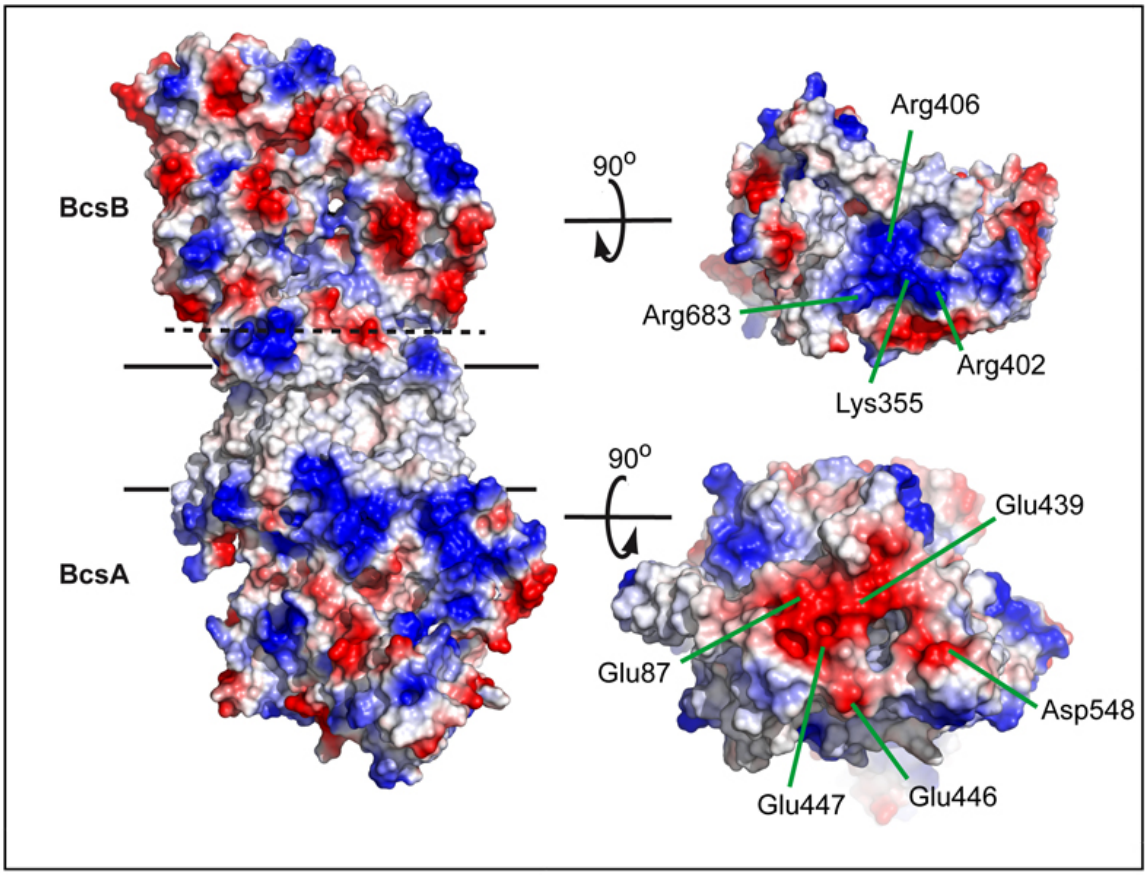
*Values in parentheses refer to the highest-resolution shell.

Supplementary Figures

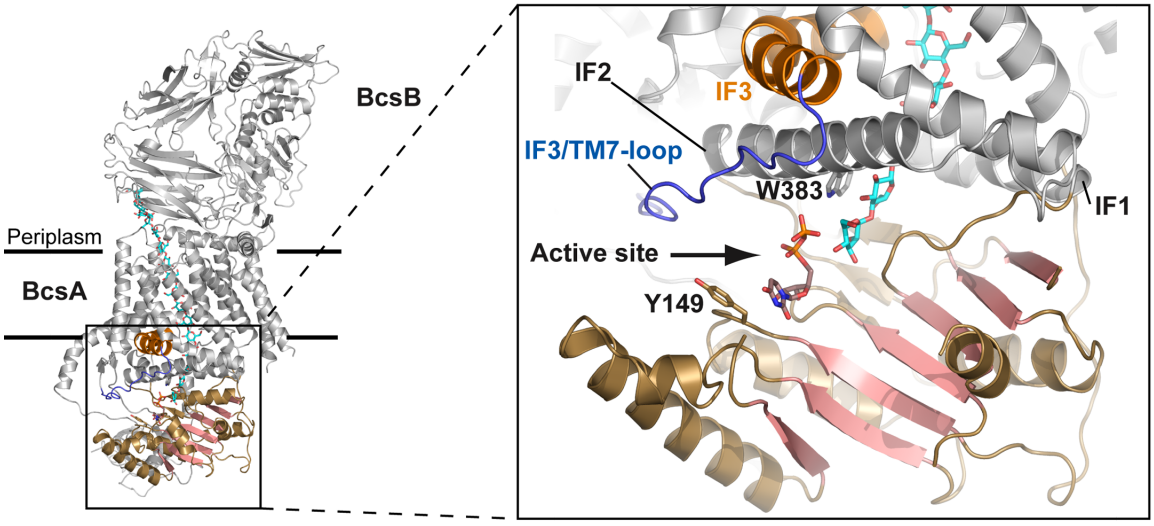




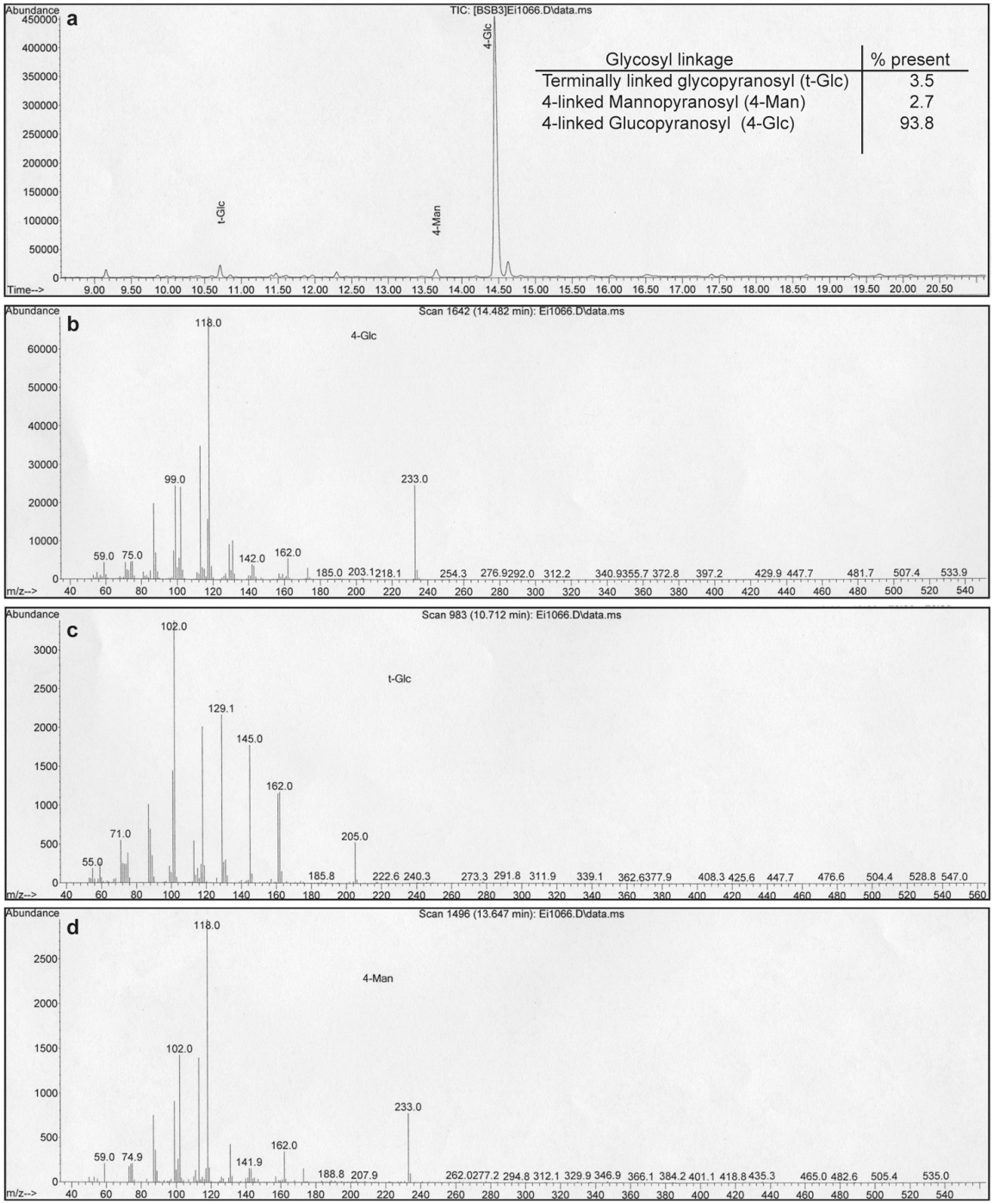
Supplementary Figure 1| Sequence alignment of *Rhodobacter sphaeroides* BcsA and *Arabidopsis thaliana* CESAs. Residues 1 to 580 of *R. sphaeroides* (Rs) BcsA are aligned with the transmembrane (TM)- and glycosyltransferase domains of *A. thaliana* (At) CESA1, 3 and 6. BcsA's C-terminal PilZ-domain was omitted from the alignment. The TM- and cytoplasmic interface helices of BcsA are shaded green and blue, respectively. The predicted CESA TM-helices are indicated with a black dashed line above the alignment. The locations of the dominant negative *thanatos* mutation (Pro578Ser) and the quinoxyphen and isoxaben resistance conferring mutations (Ala903Val and Thr942Ile) in *At* are framed blue and are indicated with the symbols "&", "✱" and "#" above the alignment, respectively. TM1 and 2 of BcsA are not present in eukaryotic CESAs and are indicated with a green box. We note that TM5 of CESA aligns with IF3 of BcsA and CESA TM7 partially aligns with the periplasmic loop connecting BcsA's TM7 and 8. Additional experiments are required to determine whether these predicted TM-helices indeed span the membrane or form interface helices on the intra- and extracellular sides of the membrane.



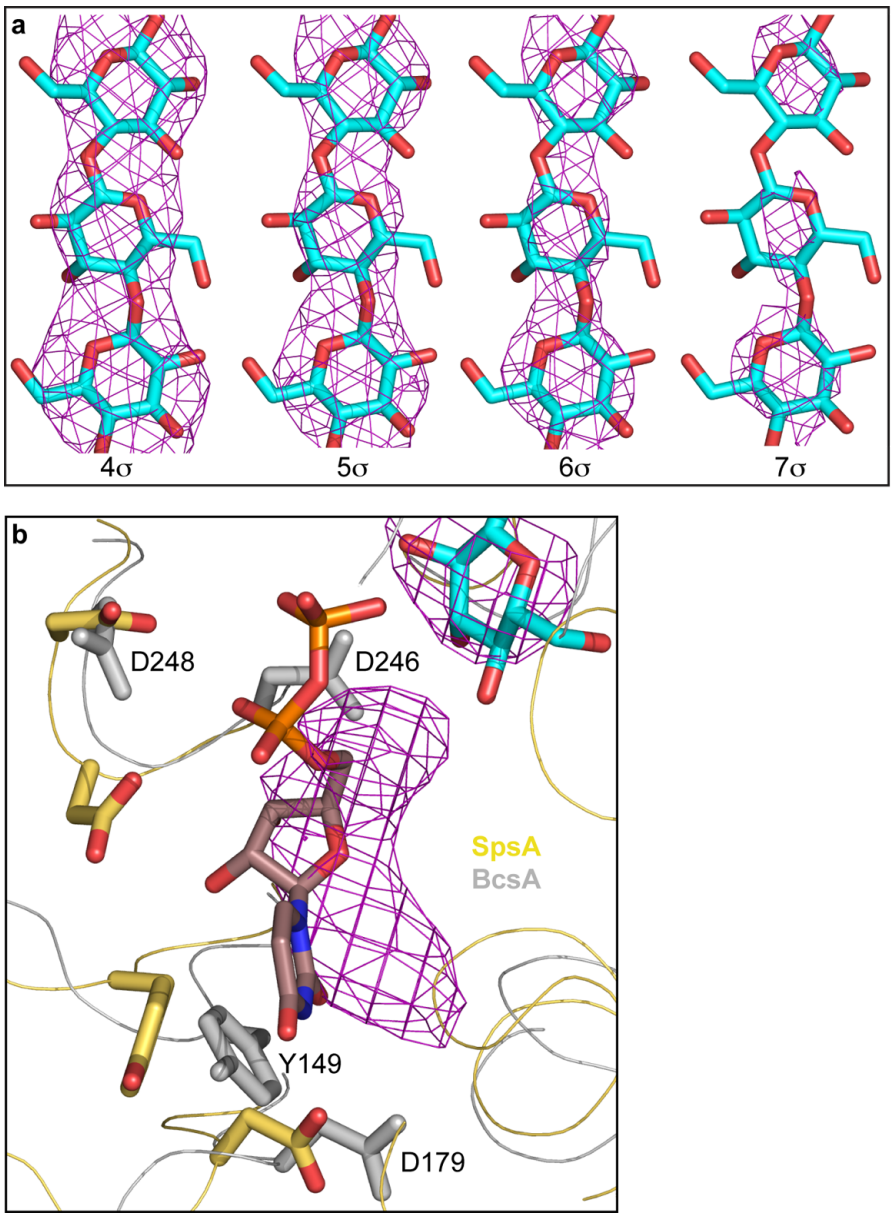
Supplementary Figure 2| Vacuum electrostatics of BcsA-B. The interaction of BcsA and BcsB is stabilized by a 4500Å^2 large interface that includes a cluster of negatively and positively charged residues on BcsA and BcsB, respectively. In addition, the TM-helix of BcsB interacts with TM-helices 1 to 3 of BcsA. The dashed line indicates the periplasmic BcsA-B interface shown on the right for the individual subunits. Horizontal bars indicate the membrane boundaries.



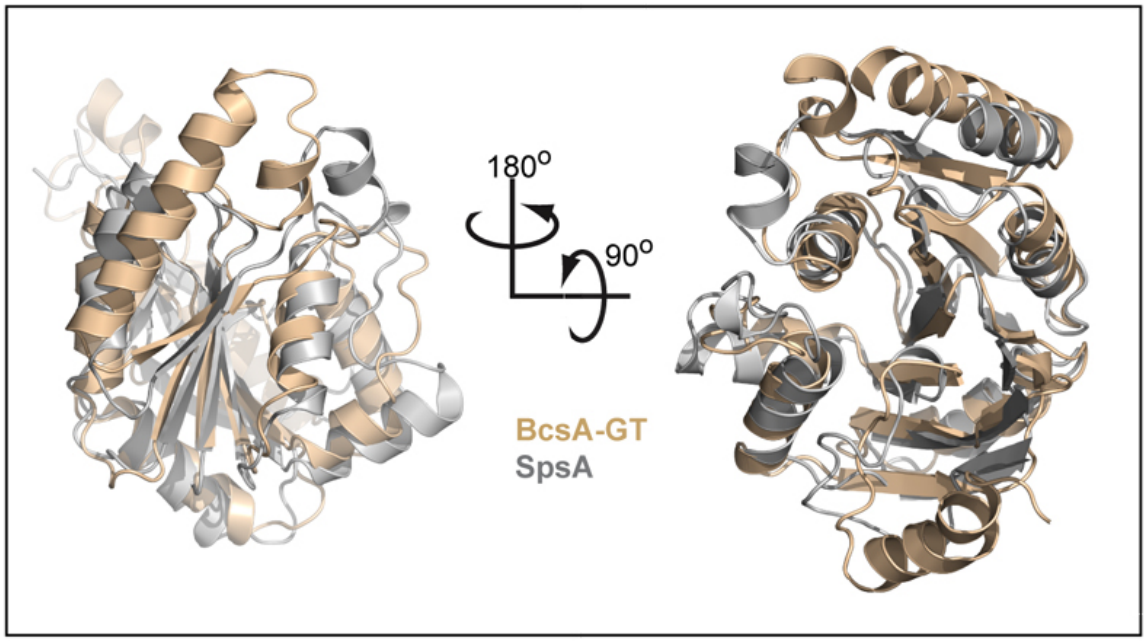
Supplementary Figure 3| The glycosyltransferase domain of BcsA. The loop connecting BcsA's TM4 and -5 (colored sand and light red) adopts a GT-A fold and forms the catalytic domain of the synthase. Access to the active site is likely controlled by the position of the conserved IF3/TM7-loop (shown in blue). Residues 223-238 and 334-354 of the GT-domain are omitted for clarity, UDP and the translocating glucan are shown as violet and cyan sticks, respectively. Trp383 of the "Q(Q/R)xRW" motif and Tyr149 are shown as sticks.



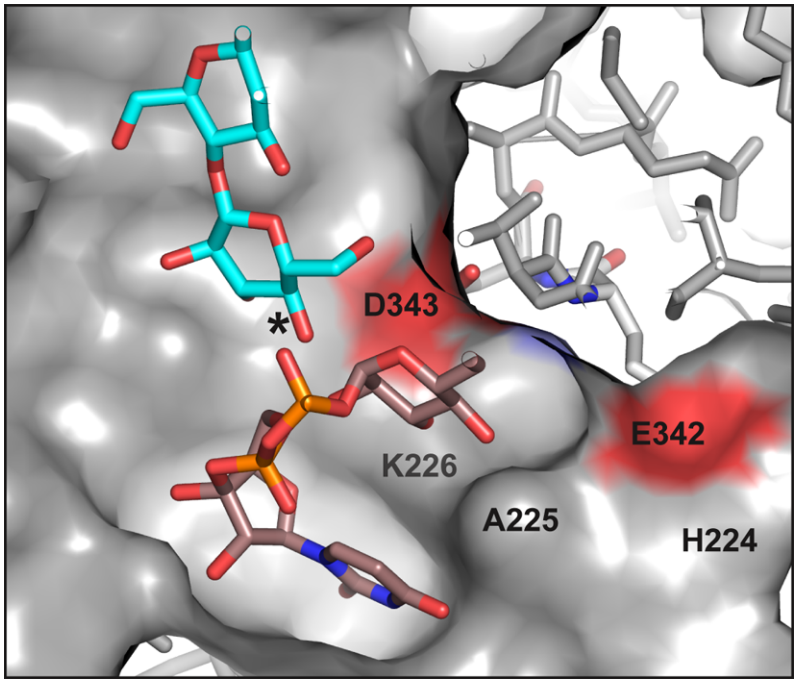
Supplementary Figure 4| Glycosyl linkage analysis of *in vitro* synthesized cellulose. The detergent solubilized *Rhodobacter* BcsA-B complex was incubated with UDP-Glc and cd-GMP at 37^{o}C. The synthesized polymer was sedimented, washed and subjected to linkage analysis by the Complex Carbohydrate Research Center, University of Georgia. The analysis involved permethylation, depolymerization, reduction and acetylation of the polymer followed by coupled gas chromatography (a) and mass spectrometry (b-d) to identify the partially methylated alditol acetates.[1]



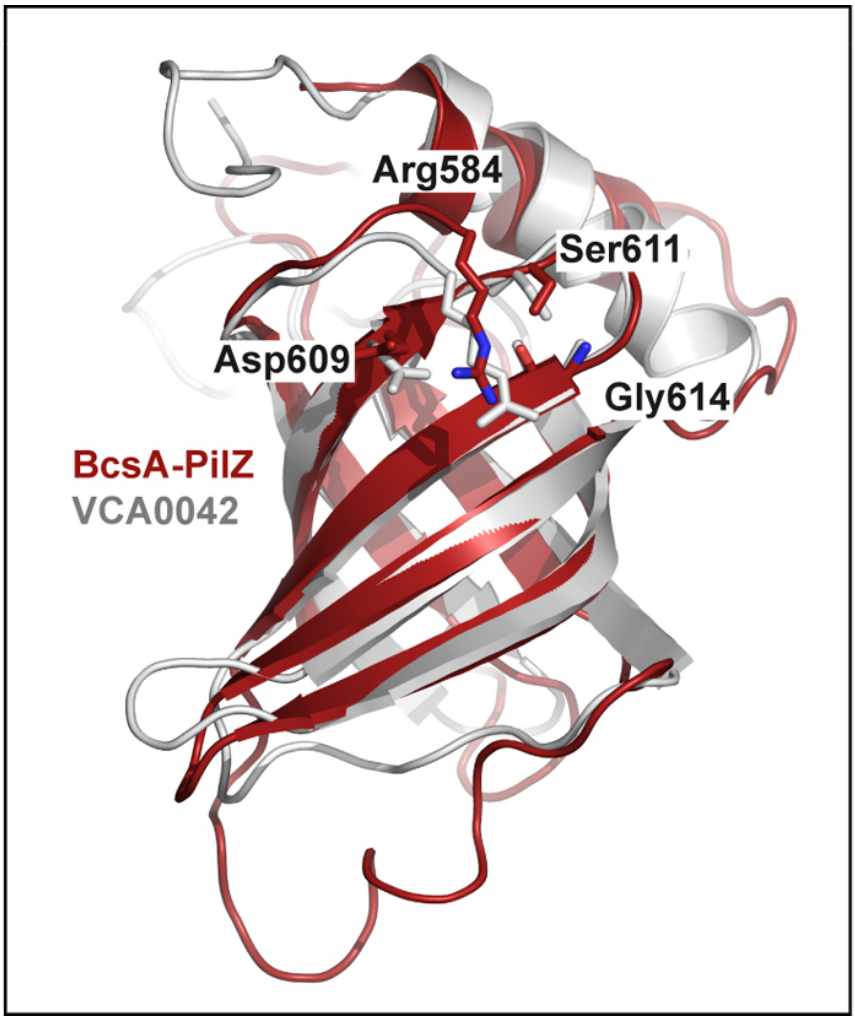
Supplementary Figure 5| Difference Fourier electron density of the translocating glucan and UDP. a, The glucose units of the translocating glucan occupy distinct positions along the translocation path. The unbiased, positive FoFc-difference Fourier electron density was contoured at the indicated levels and reveals the position of the individual glucose units. The density was calculated with phases obtained from a BcsA-B model prior to placing and refining the glucan. **b**, BcsA was superimposed with the UDP-bound structure of SpsA (pdb entry 1QGS). BcsA is shown in grey, SpsA is colored yellow and the SpsA-bound UDP molecule is shown as violet sticks. Conserved residues coordinating UDP in SpsA and BcsA are shown as sticks and are labeled for BcsA. The coordination of the hook-shaped density and the good agreement with the SpsA-UDP complex support its interpretation as a weakly bound UDP molecule.



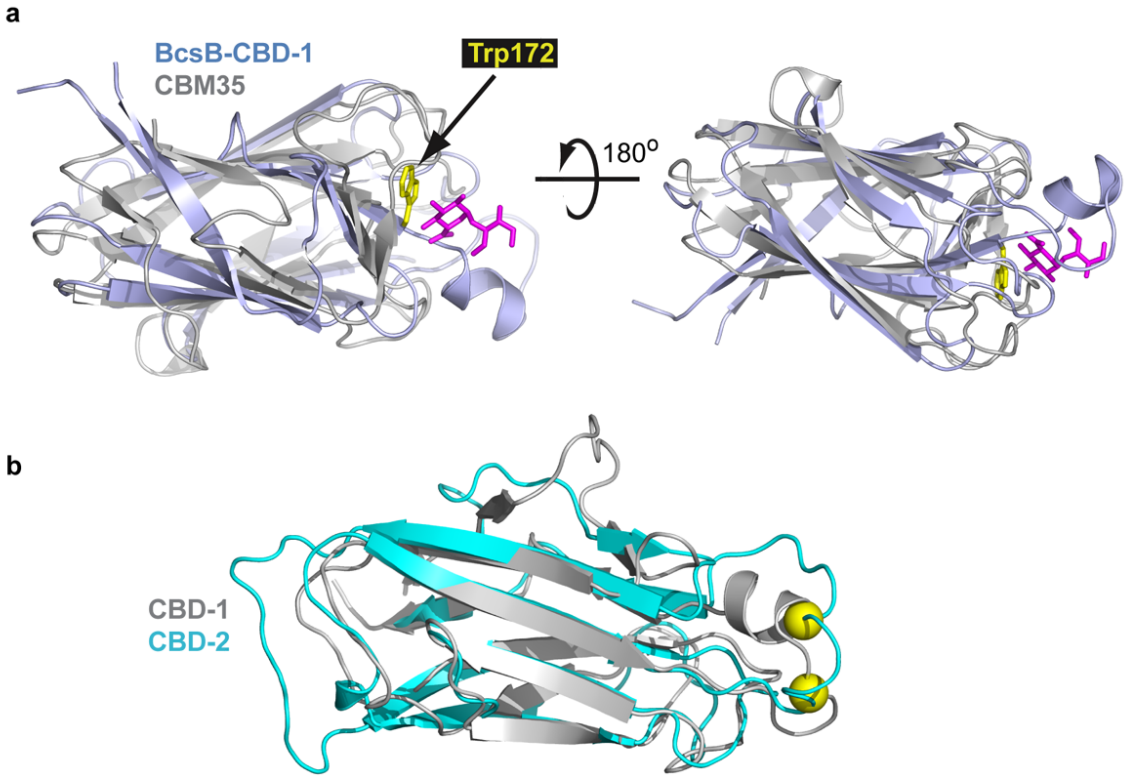
Supplementary Figure 6| Superimposition of BcsA's glycosyltransferase domain with SpsA. Residues 142 to 367 of BcsA were superimposed with residues 2 to 217 of SpsA (pdb entry 1QGS) in Coot by secondary structure matching, SSM. Both proteins adopt a GT-A fold and align with an r.m.s.d. of 2.15Å between Cα atoms.



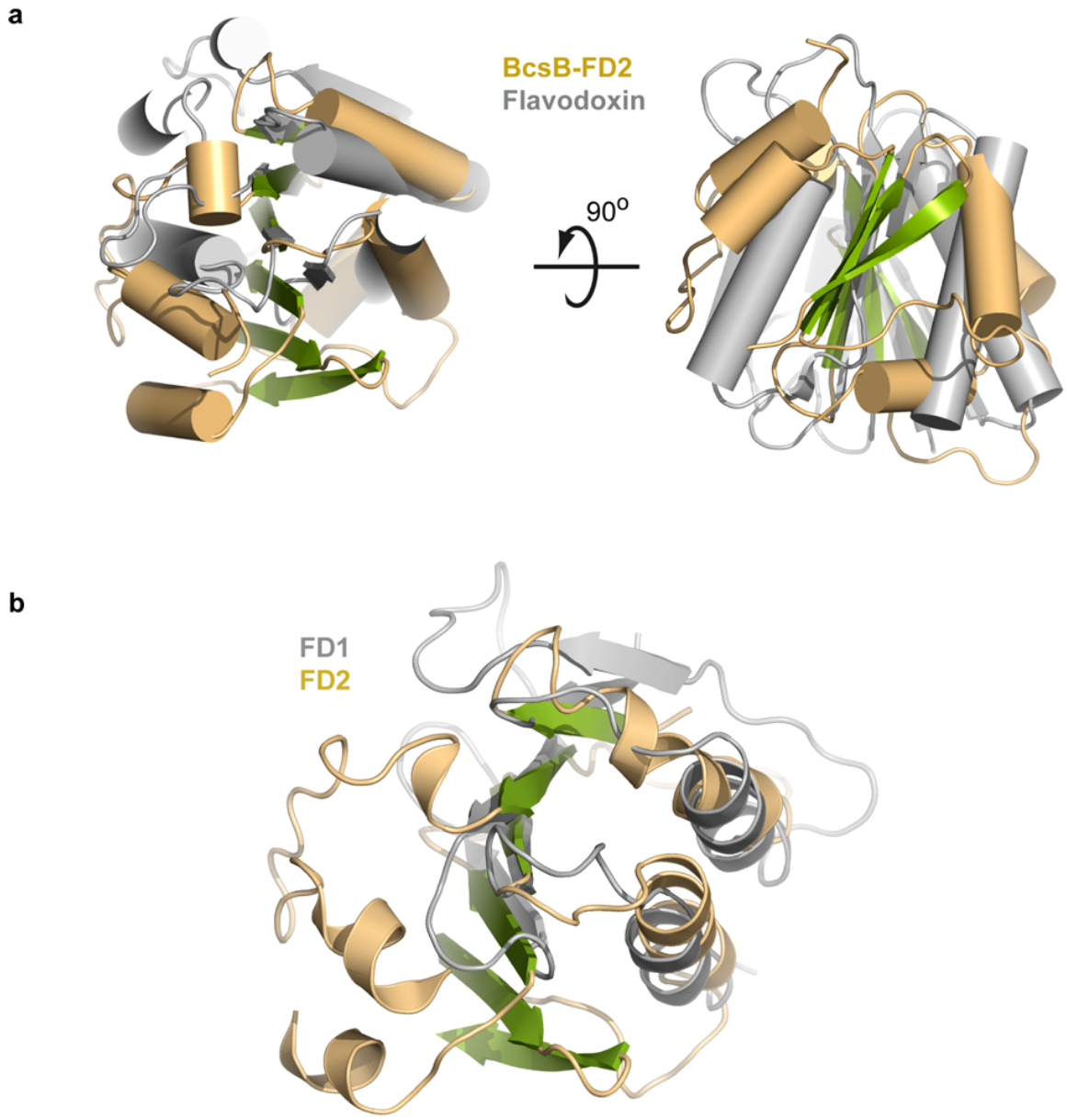
Supplementary Figure 7| Proposed donor glucose binding site. Surface representation of BcsA's GT-domain. Residues 224-226 of the "HAKAGN" and 341-343 of the "TED" motifs might form a binding site for the donor Glc next to the UDP binding pocket. This would position the β-face of the anomeric carbon towards the 4' hydroxyl of the acceptor (✱). The acceptor glucan and UDP-Glc are shown as cyan- and violet sticks. UDP-Glc was manually docked into the binding pocket.



Supplementary Figure 8| Superimposition of BcsA's PilZ-domain with the VCA0042 protein. Residues 584 to 693 of BcsA were superimposed with residues 139 to 238 of the cyclic-di-GMP binding protein VCA0042 (pdb entry 3KYG) in Coot by secondary structure matching, SSM. The Cα atoms of both β-barrels align with an r.m.s.d. of 2.2Å. Conserved residues of BcsA likely implicated in cyclic-di-GMP binding are labeled and shown as sticks.



Supplementary Figure 9| Superimposition of BcsB's CBD-1 with the carbohydrate binding module family 35. a, Residues 54 to 187 of BcsA were superimposed with the carbohydrate binding module 35 from *Cellvibrio japonicus* (pdb entry 2W87) in Coot by secondary structure matching, SSM. The glucuronic acid disaccharide observed in pdb 2W87 is shown as pink- and the conserved Trp172 of BcsB as yellow sticks. The disaccharide in 2W87 makes CH-π interactions with a Trp residue. **b**, Superimposition of BcsB's CBD-1 and -2. Conserved cysteines forming a disulfide bond between CBD-1 and -2 are indicated with a yellow sphere for their Cα atoms.



Supplementary Figure 10| BcsB's FD-domains adopt a flavodoxin fold. a, Flavodoxin from *Desulfovibrio desulfuricans* (pdb entry 3KAP) was aligned with FD2 of BcsB. The structures share a 4-stranded β-sheet that is framed by two α-helices on either side. **b**, Superimposition by secondary structure matching of BcsB's FD1 and -2.

Supplementary Reference

1. York, W. S., Darvill, A. G., McNeil, M., Stevenson, T. T. & Albersheim, P. Isolation and Characterization of Plant Cell Walls and Cell Wall Components. *Methods Enzymol* **118**, 1-38 (1986).