Supporting Information

Cellulose Structural Polymorphism in Plant Primary Cell Walls Investigated by High-Field 2D Solid-State NMR Spectroscopy and Density Functional Theory Calculations

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Table S1. Structural parameters of non-equivalent glucose units used in the DFT calculation. U and D annotate the two non-equivalent glucose units within the same glucan chain in the Iα allomorph, while C and O denote to center and origin chains of the Iβ allomorph. Apostrophe denotes the atoms in the adjacent glucose residue.

		H---C distance (Å)		H---H distance (Å)			Torsion angles (°)					
		H_{6a}-C_4	H_{6b}-C_4	H_{6a}-H_4	H_{6b}-H_4	H'_1-H_4	$\theta(H_{6a}$-C_6 •C_4-$H_4)$	$\theta(H_{6b}$-C_6 •C_4-$H_4)$	$X_1(O_5$-C_5 -C_6-$O_6)$	$X_2(C_4$-C_5 -C_6-$O_6)$	Φ(C5-C4-O4-C1′)	Ψ (C4-O4-C1′-O5′)
I_β tg	C	2.7	3.5	2.4	3.7	2.1	0.7	114.2	167.9	-74.3	-143.2	-92.7
	O	2.7	3.5	2.5	3.7	2.2	-6.6	106.7	165.1	-76.4	-145.0	-94.2
I_β gt	C	2.8	2.8	3.0	2.5	2.1	-101.9	6.3	58.1	178.1	-145.1	-93.2
	O	2.9	2.8	3.2	2.6	2.1	-110.8	-2.2	52.1	170.4	-150.7	-92.2
I_β gg	C	3.5	2.8	3.7	3.0	2.1	137.8	-99.6	-63.9	57.2	-140.5	-86.9
	O	3.5	2.9	3.8	3.3	2.1	115.5	-115.9	-67.3	54.0	-155.7	-85.4
I_α tg	U	2.7	3.5	2.4	3.6	2.1	-3.3	109.3	165.3	-76.8	-143.1	-92.6
	D	2.7	3.5	2.4	3.7	2.2	-5.7	107.8	165.2	-76.0	-145.6	-95.0
I_α gt	U	2.8	2.7	3.1	2.5	2.2	-109.6	-2.6	58.4	177.0	-156.1	-89.4
	D	2.7	2.9	2.9	2.7	2.2	-96.1	12.8	77.2	-163.9	-155.9	-85.4
I_α gg	U	3.5	2.8	3.7	3.1	2.2	130.4	-107.4	-62.7	57.8	-140.0	-81.3
	D	3.5	2.8	3.8	3.2	2.2	118.4	-116.4	-67.9	52.1	-160.2	-92.9

Table S2. Estimated fractions of different glucose conformations in crystalline Iα (A and A') and Iβ (B and B') allomorphs. The estimation is based on spectral deconvolution results of Kono and Numata [1], with an additional assumption that A and A' have equal populations.

Cellulose	Organism	*A*	*A'*	*B*	*B'*
Iα dominant	*Cladophora*	32%	32%	17%	18%
Iβ dominant	tunicate	11%	11%	38%	41%

Table S3. ^{13}C chemical shift RMSDs (ppm) between measured primary wall cellulose and DFT calculated chemical shifts for different crystalline allomorphs, hydroxymethyl conformations, and glucose residue location. C and O denote center and origin sheets in the Iβ allomorph while U and D denote the two non-equivalent glucose units in an Iα chain. The five types of crystalline cellulose signals observed in this study are denoted as *a, b, c, d, e*.

		a	*b*	*c*	*c*	*e*
Iβ allomorph	gg_C	5.5	5.3	5.2	4.7	5.8
	gg_O	4.5	4.5	4.4	3.8	4.9
	gt_C	4.3	4.0	3.9	3.6	4.3
	gt_O	2.3	2.5	2.5	1.9	2.7
	tg_C	2.4	2.6	2.0	2.1	2.4
	tg_O	1.9	2.3	2.0	1.8	2.1
Iα allomorph	gg_U	5.3	5.2	5.0	4.5	5.6
	gg_D	5.6	5.5	5.5	4.9	6.0
	gt_U	4.3	4.3	4.4	3.6	4.7
	gt_D	4.1	3.8	3.7	3.5	4.2
	tg_U	2.0	2.2	1.9	1.7	2.2
	tg_D	2.1	2.5	2.0	2.2	1.9

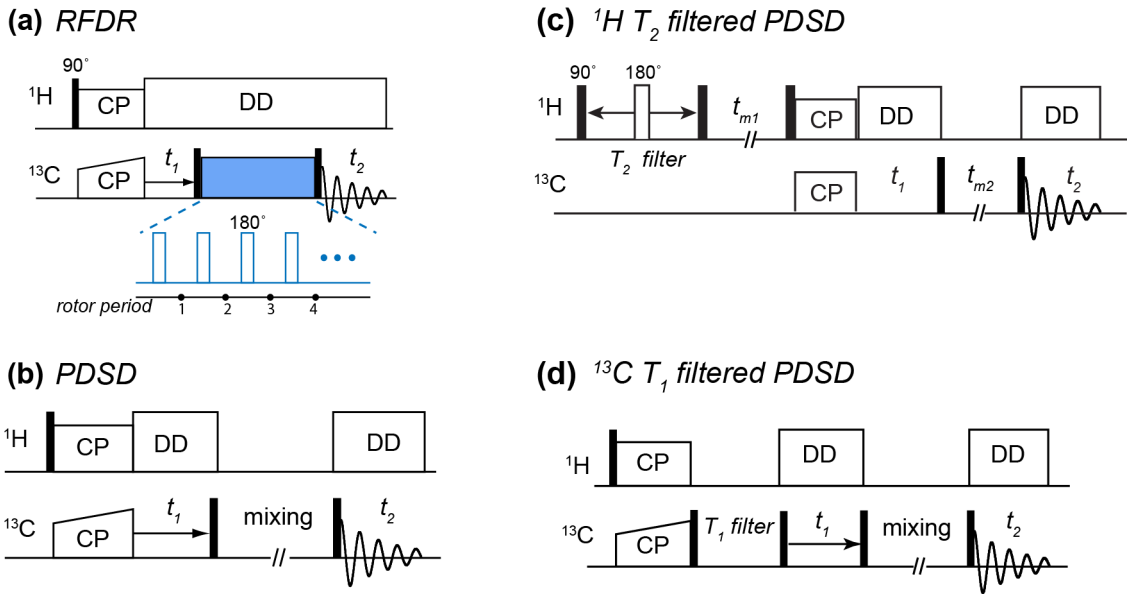

Figure S1. Different 2D ^{13}C-^{13}C correlation pulse sequences used to measure cellulose chemical shifts, structure, and dynamics. (a) Radio-frequency-driven recoupling (RFDR) experiment. (b) Proton-driven ^{13}C spin diffusion (PDSD) experiment. (c) ^{1}H T_2 filtered PDSD experiment for determining the water contact of wall polysaccharides. (d) ^{13}C T_1 filtered PDSD experiment for measuring the ^{13}C T_1 relaxation rates of wall polysaccharides.

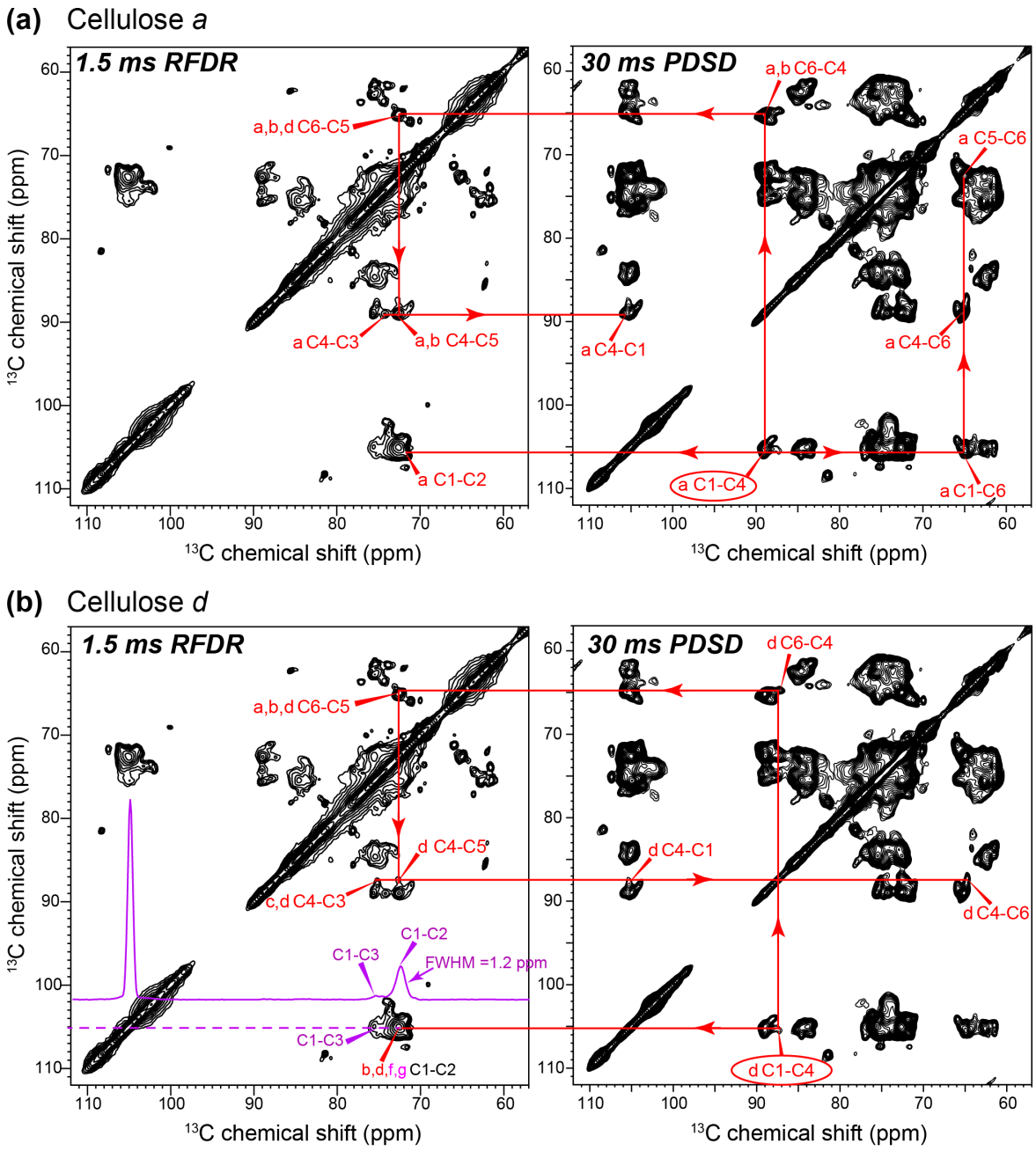

Figure S2. Assignment of (a) cellulose *a* and (b) cellulose *d* ^{13}C chemical shifts of the *Brachypodium* cell wall using 2D RFDR and PDSD spectra. The C1-C4 cross peaks serve as the starting points for assignment.

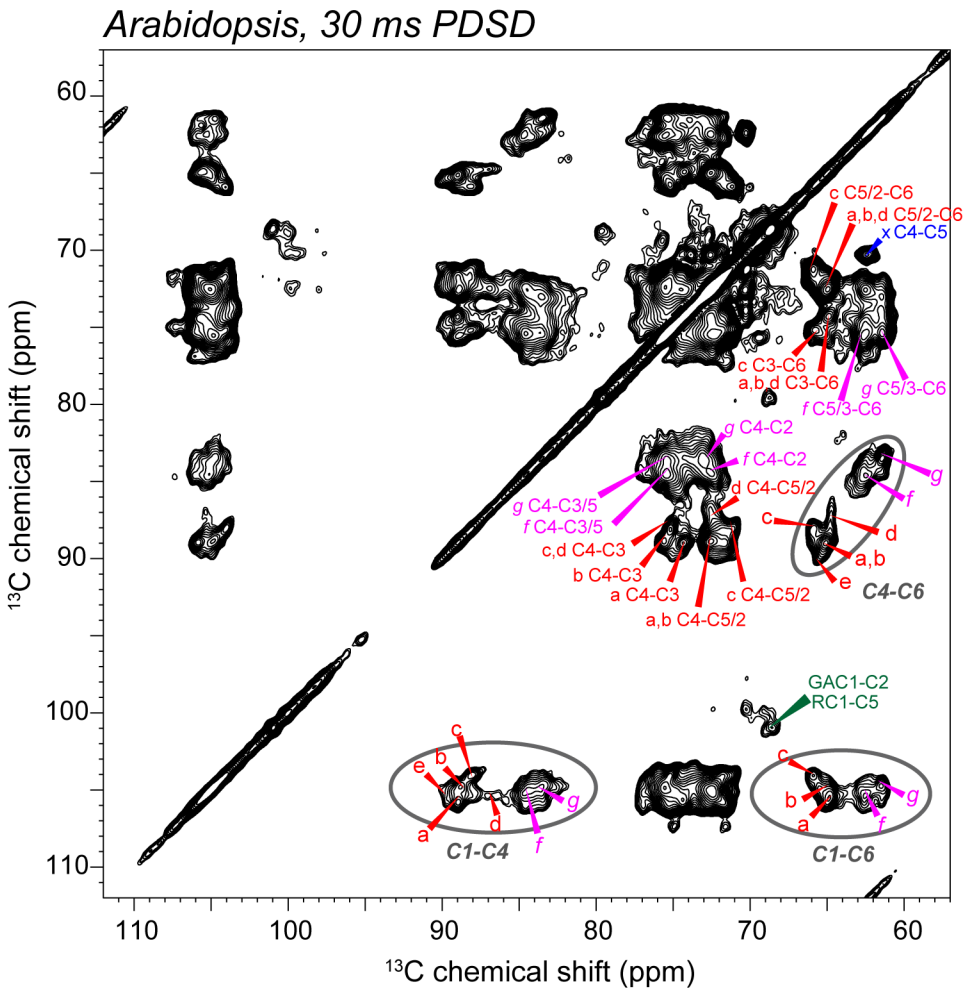

Figure S3. 2D ^{13}C-^{13}C PDSD spectrum of the *Arabidopsis* cell wall. The spectrum was measured with 30 ms mixing at 296 K on an 800 MHz spectrometer. The characteristic C1-C4, C1-C6 and C4-C6 cross peaks are highlighted.

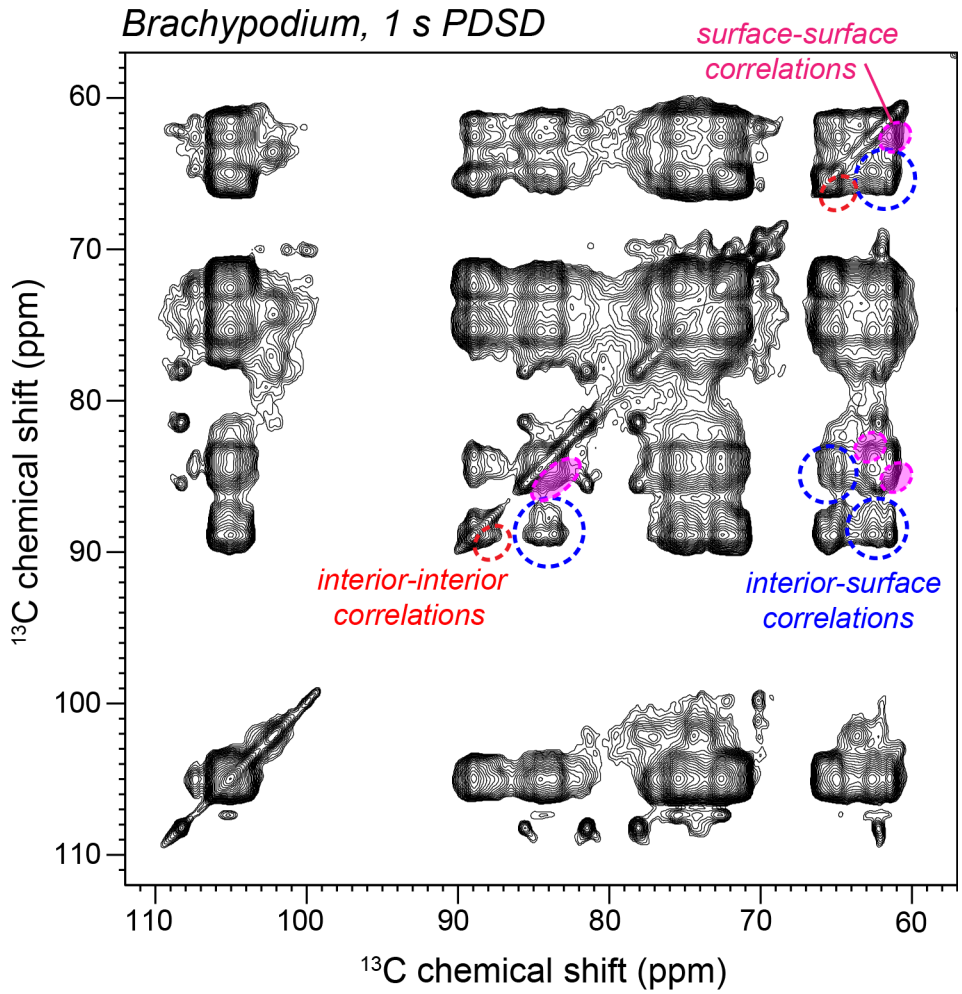

Figure S4. 2D PDSD spectrum of the *Brachypodium* cell wall with 1 s mixing. The spectrum was measured at 296 K on an 800 MHz spectrometer. Regions of long-range cross peaks are highlighted.

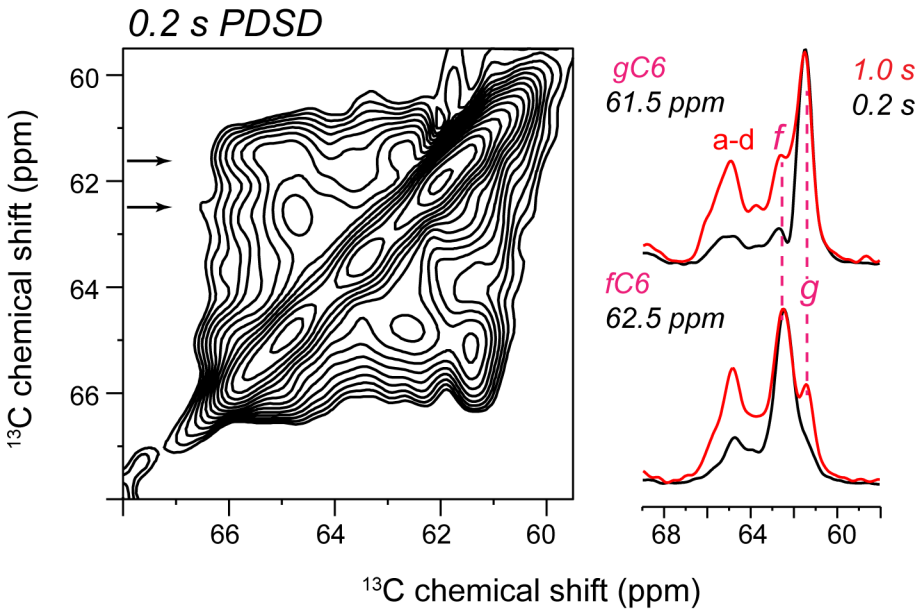

Figure S5. 0.2 s PDSD spectrum of the *Brachypodium* cell wall measured on an 800 MHz spectrometer. The surface-surface and surface-interior cellulose cross peaks show similar intensities.

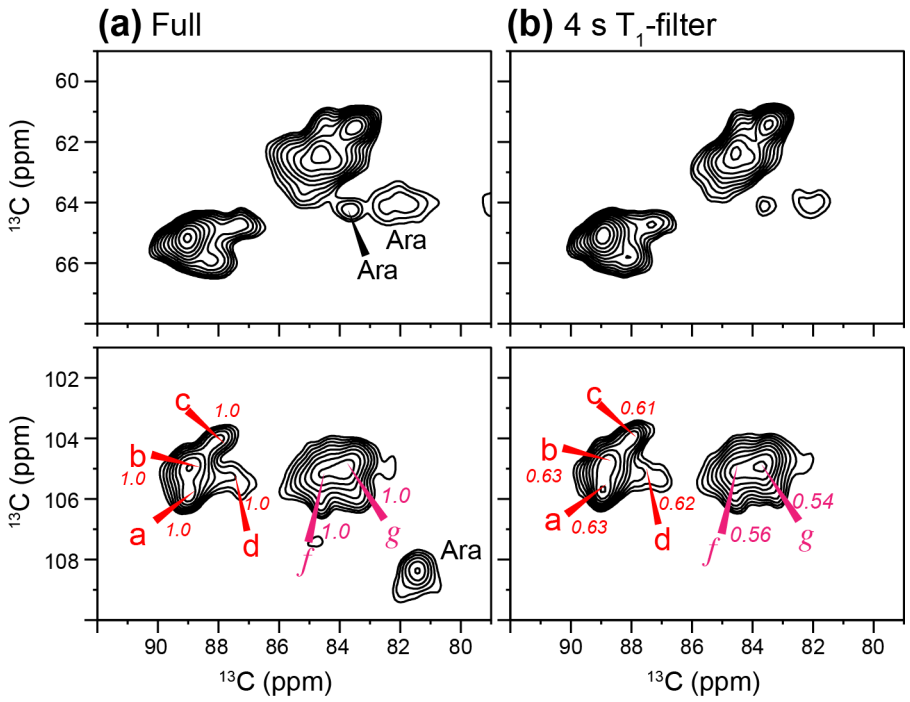

Figure S6. ^{13}C T_1 filtered 2D PDSD spectra of the *Brachypodium* primary cell wall. (a) Full 2D spectrum. (b) 2D spectrum with a 4 s T_1 relaxation filter. The relative intensity of each cellulose cross peak is indicated. Interior cellulose signals decay to ~62% while surface cellulose signals decay to ~55%, indicating that surface cellulose is slightly more dynamic on the nanosecond timescale.

Supplementary References

1. Kono, H.; Numata, Y., Structural investigation of cellulose I_α and I_β by 2D RFDR NMR spectroscopy: determination of sequence of magnetically inequivalent D-glucose units along cellulose chain. *Cellulose* **2006,** *13*, 317-326.