Supporting Information

Pectin-Cellulose Interactions in *Arabidopsis* Primary Cell Wall by Two-Dimensional Magic-Angle-Spinning Solid-State NMR

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Table S1. Two sets of interior cellulose ^{13}C chemical shifts (ppm) resolved at the 900 MHz NMR.

Cellulose	C1	C2	C3	C4	C5	C6
Interior 1	105.1	72.2	74.1	88.6	72.2	64.7
Interior 2	104.1	72.2	75.0	87.9	70.8	65.6

Table S2. ^{13}C chemical shifts (ppm) of assigned protein residues in *Arabidopsis* primary cell wall. The ^{13}C chemical shifts are referenced to neat TMS.

Residue	CO	Cα	Cβ	Cγ	Cδ	Cε	Cζ
Gly	171.9 173.3	43.5 43.5					
Hyp				72.5	58.6		
Lys			33.7		27.5	40.1	
Pro		60.0	30.7	25.7	48.2		
Ala		48.6 50.1 52.2	21.1 17.4 16.7				
Tyr		54.4	37.8 42.0	133.8	131.1	115.3	157.2/155.8 157.2
Phe		55.1	36.7	137.0	129.2	129.2	127.0
Ser		55.9	62.2				
Thr		58.8	67.5	19.7			
Ile		57.3	38.8 35.6	26.1	15.6 15.6	12.6	
Leu		55.3 51.2	40.1 41.5	25.3 24.3	 21.1		
Val			32.7 29.9	19.3 19.3			

Table S3. Spin diffusion buildup time constants of polysaccharide cross peaks in primary cell wall.

(ω_1, ω_2) (ppm)	Assignment	τ_1 (ms)	τ_2 (ms)
Interior-interior cellulose			
(89, 65)	iC4 – iC6	10±1.9	
(65, 89)	iC6 – iC4	29±45	
(89, 72)	iC4 – iC5/iC2, s/G/x/L C2, GA/R C3, Gal/GA C5	5.4 ± 1.7	
(65, 105)	iC6 – i/s/Gal/G/L C1	27±3.5	
(65, 75)	iC6 – iC3, sC3/5, Gal C2/3, GC3/5, LC5, x C3	15±1.7	
Interior-surface cellulose			
(89, 84)	iC4 – s/G/A C4	8.1±20 (24%)	150±94 (76%)
(89, 62)	iC4 – s/G/Gal C6, A/x C5	12±14 (37%)	230±130 (63%)
(65, 84)	iC6 – s/G/A C4	6.3±3.1 (58%)	460±380 (42%)
Surface-interior cellulose			
(84, 89)	s/G/A C4 – iC4	3.4±2.2 (34%)	190±53 (66%)
(84, 65)	s/G/A C4 – iC6	2.0±1.6 (27%)	150±24 (73%)
(62, 89)	s/G/A C6 – iC4	3.5±2.9 (23%)	250±50 (77%)
(84, 62)	s/G/A C4 – s/G/Gal C6, A/x C5	12±4.2	
Interior-pectin cellulose			
(89, 101)	iC4 – HGA/R C1		200±90
(89, 82)	iC4 – A C2	29±11	
(89, 99)	iC4 – GA/X C1		350±100
(89, 69)	iC4 – residual iC5, GA C2, R C5	6.7±2.6	
(65, 101)	iC6 – HGA/R C1	5.2±1.1	
(65,105)	iC6 – i/s/Gal/G/L C1	27±3.5	
(65, 69)	iC4 – GA C2, R C5		
(65, 99)	iC4 – GA/X C1	7.5±1.2	
Pectin-interior cellulose			
(99, 89)	GA/Xyl C1 – iC4		370±190
(82, 89)	A C2 – iC4	1.0±6.1 (37%)	510±170 (63%)
(101, 65)	HGA/Rha C1 – iC6	11±11 (44%)	490±550 (56%)
Surface-pectin cellulose			
(84, 101)	s/G/A C4 – HGA/R C1	18±3.5	
(84, 99)	s/G/A C4 – GA/X C1	14±2.0	
(84, 69)	s/G/A C4 – GA C2, R C5	1.8±0.70 (64%)	470± 180(36%)
Pectin-surface cellulose			
(101, 84)	HGA/R C1 – s/G/A C4	16±5.8 (69%)	540±720 (31%)
(99, 84)	GA/X C1 – s/G/A C4	3.6±2.9 (39%)	350±170 (61%)
(80, 84)	GA C4/R C2 – s/G/A C4	17±23	
Pectin-pectin			
(101, 80)	HGA/R C1 – GA C4/ R C2	13±2.6	

(101, 69)	HGA/R C1 – GA C2/ R C5	4.0±0.7	
(80, 101)	GA C4/ R C2 – HGA/R C1	14±1.7	
(69, 101)	GA C2/ R C5 – HGA/R C1	4.0±0.6	
(101, 54)	HGA/R C1– OCH_3		160±40
(54, 101)	OCH_3 – HGA/R C1		160±25

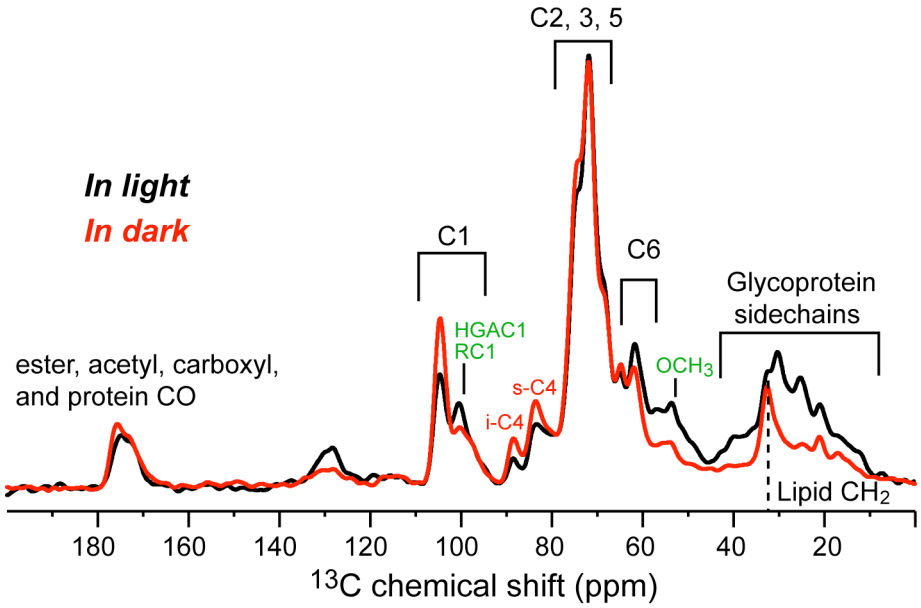

Figure S1. 1D ^{13}C CP-MAS spectra of unlabeled *Arabidopsis* primary cell walls obtained from plants grown in dark (red) versus light (black). The spectra were measured at 253 K under 6 kHz MAS on a 400 MHz NMR spectrometer. Note the similarity of the polysaccharide region of the spectra from 60-110 ppm. Main differences are that the dark-grown cell wall has less protein, less methyl ester (53.5 ppm peak), and slightly less pectins relative to cellulose, as manifested by the weaker 99-ppm peak relative to the 89-ppm peak.

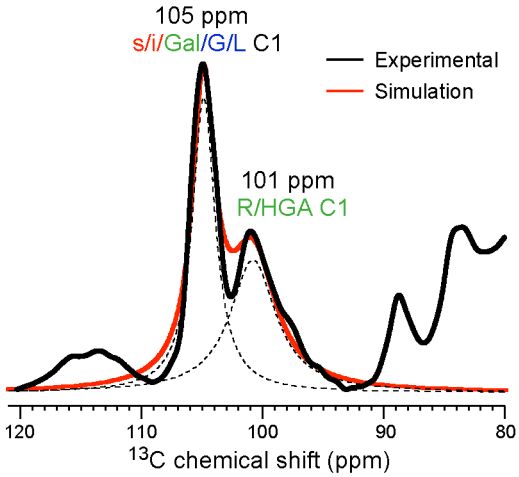

Figure S2. Deconvolution of the 105-ppm and 101-ppm peaks in the 1D ^{13}C CP-MAS spectrum shown in Figure 1. Black: experimental spectrum. Red: sum of the deconvoluted peaks. The deconvolution shows that the upfield wing of the 105-ppm peak contributes only 10% of the total intensity at 101 ppm. For the 105- and 101-ppm peaks, the full width at half maximum are 2.3 and 4.6 ppm, respectively, the relative height is 1 : 0.45 and the relative area is 1 : 0.9.

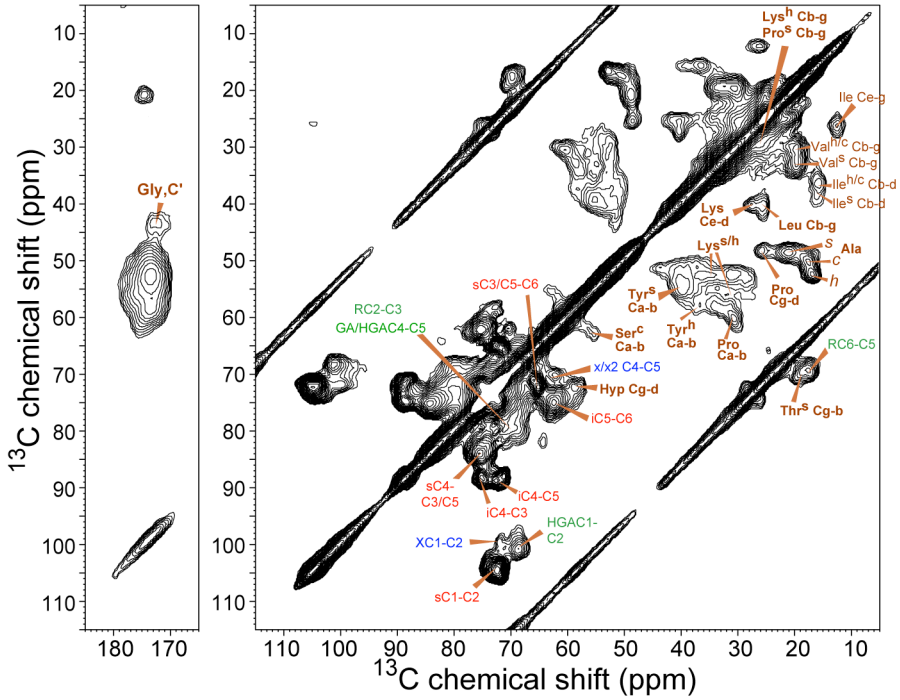

Figure S3. 2D ^{13}C DQ filtered spectrum of ^{13}C-labeled primary cell wall. A Hyp Cγ-Cδ cross peak at (72, 59) ppm is detected.

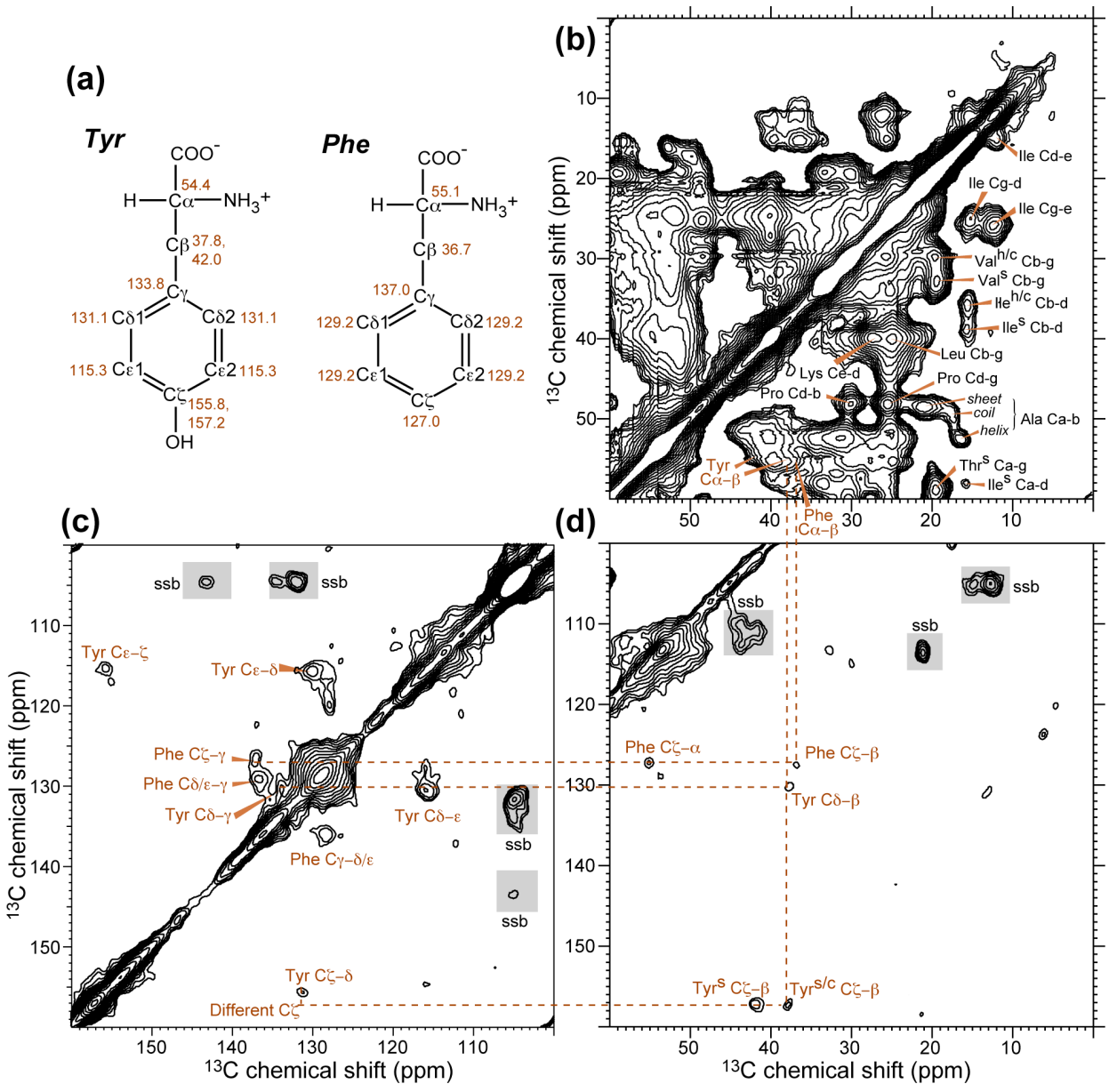

Figure S4. Assignment of Tyr and Phe chemical shifts from the 30-ms ^{13}C PDSD spectrum. (a) Summary of the assigned ^{13}C chemical shifts. (b) Aliphatic region of the 2D spectrum. The area containing the Cα-Cβ peaks of Tyr and Phe shows significant overlap. (c) Aromatic region of the 2D spectrum, showing the Tyr and Phe spin systems. (d) Correlation between aromatic and aliphatic carbons, where two Tyr Cζ-Cβ peaks are observed.

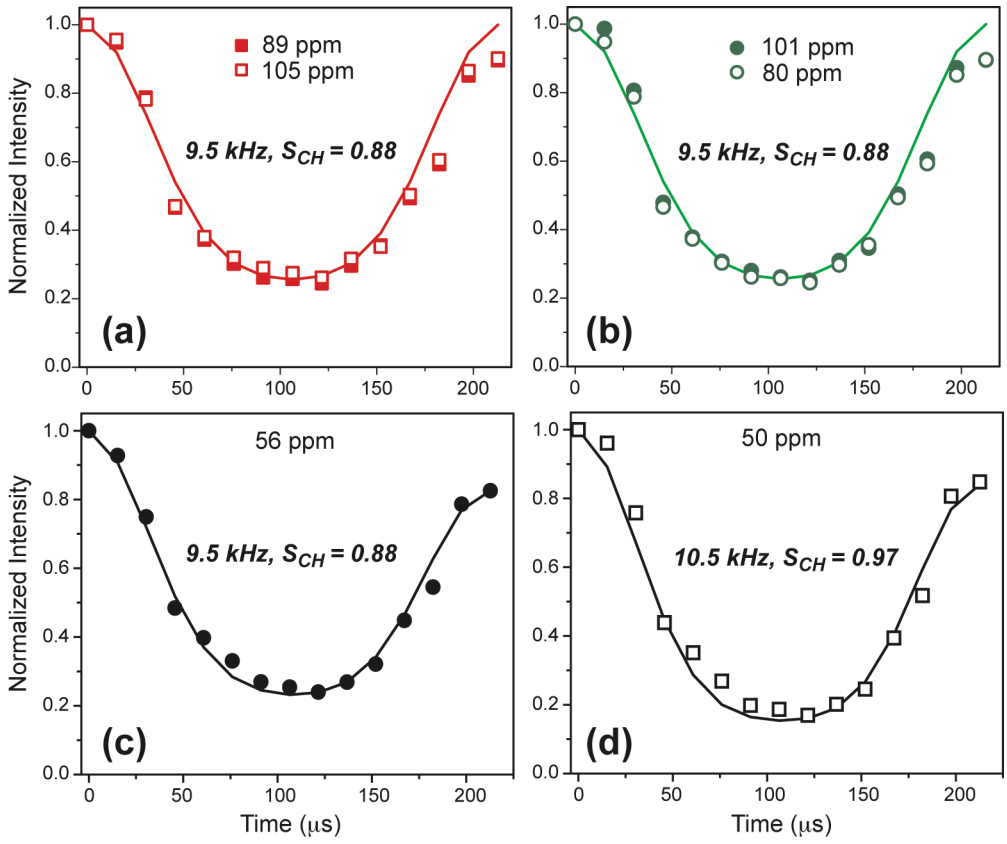

Figure S5. ^{13}C-^{1}H DIPSHIFT spectra of the cell wall at 253 K under 4.7 kHz MAS. (a) Cellulose-rich peaks. 89 ppm: iC4; 105 ppm: i/s/G/Gal C1. (b) Predominantly pectin peaks. 101 ppm: Rha and HGA C1; 80 ppm: Rha C2 and GA C4. (c), (d) Typical protein Cα peaks. 56 ppm: Ser/Phe/Leu Cα; 50 ppm: Ala Cα. For panels (c) and (d), the experimental data show moderate T_2 relaxation, which was simulated using a single exponential decay. The figure shows that cellulose and pectins have similar order parameters S_{CH} of 0.88, indicating similar amplitudes of motion, while the proteins are more rigid at this temperature. Therefore, lack of cross peaks must be attributed to long distances rather than molecular motion.

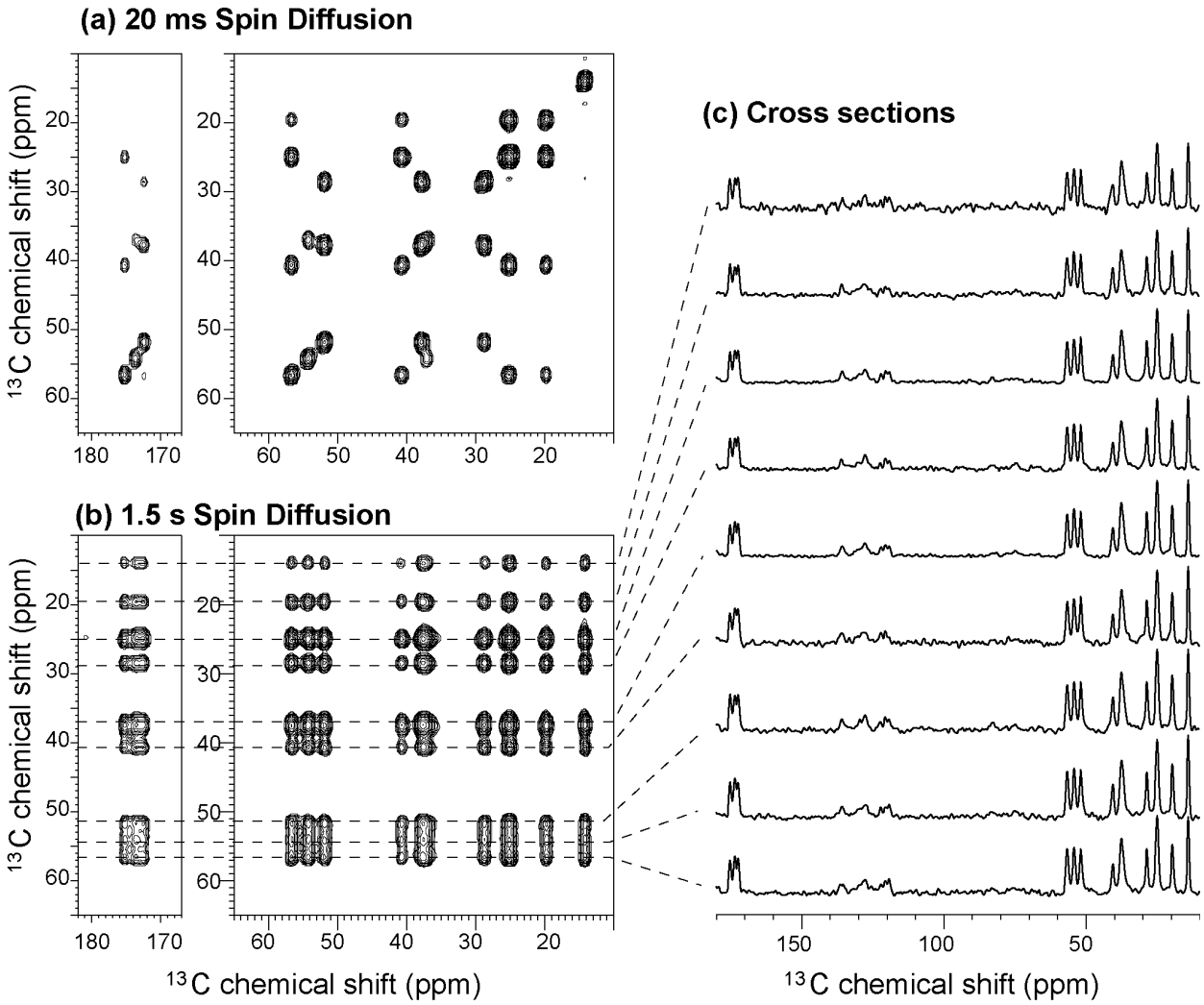

Figure S6. Complete equilibration of ^{13}C magnetization of the tripeptide formyl-Met-Leu-Phe at long spin diffusion mixing times. (a) 20 ms 2D PDSD spectrum. (b) 1.5 s 2D PDSD spectrum. (c) Cross sections of the 1.5 s 2D spectrum show identical intensity patterns for all slices.