Fast MAS $^1$H–$^{13}$C correlation NMR for structural investigations of plant cell walls

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Abstract

Plant cell walls consist of a mixture of polysaccharides that render the cell wall a strong and dynamic material. Understanding the molecular structure and dynamics of wall polysaccharides is important for understanding and improving the properties of this energy-rich biomaterial. So far, solid-state NMR studies of cell wall structure and dynamics have solely relied on $^{13}$C chemical shifts measured from 2D and 3D correlation experiments. To increase the spectral resolution, sensitivity and upper limit of measurable distances, it is of interest to explore $^1$H chemical shifts and $^1$H-detected NMR experiments for analyzing cell walls. Here we demonstrate 2D and 3D $^1$H–$^{13}$C correlation experiments at both moderate and fast MAS frequencies of 10–50 kHz to resolve and assign $^1$H chemical shifts of matrix polysaccharides in Arabidopsis primary cell walls. Both $^{13}$C-detected and $^1$H-detected experiments are implemented and are shown to provide useful and complementary information. Using the assigned $^1$H chemical shifts, we measured long-range correlations between matrix polysaccharides and cellulose using $^1$H–$^1$H instead of $^{13}$C–$^{13}$C spin diffusion, and the 2D experiments can be conducted with either $^{13}$C or $^1$H detection.

Keywords Arabidopsis · $^1$H chemical shift · Ultrafast MAS · $^1$H detection · Cellulose · Matrix polysaccharides

Abbreviations

| Abbreviation | Definition |
|--------------|------------|
| Ara          | Arabinose  |
| CW           | Cell wall  |
| CP           | Cross polarization |
| DP           | Direct polarization |
| Gal          | Galactose  |
| GalA         | Galacturonic acid |
| Glc          | Glucose    |
| HG           | Homogalacturonan |
| INEPT        | Insensitive Nuclei Enhanced by Polarization Transfer |
| i            | Interior crystalline cellulose |
| MurNac       | N-Acetyl-muramic acid |
| Man          | Mannose    |
| MAS          | Magic-angle spinning |
| RG-I         | Rhamnogalacturonan I |
| Rha          | Rhamnose   |
| SSNMR        | Solid-state nuclear magnetic resonance |
| s            | Surface amorphous cellulose |

TOCSY  Total Correlated Spectroscopy
XyG    Xyloglucan
Xyl    Xylose
Xn     Xylan

Introduction

Growing plants have a thin, pliant, and highly hydrated primary cell wall, which mainly contains three types of polysaccharides: cellulose (15–40%), hemicellulose (20–30%), and pectins (30–50%) (Carpita and Gibeaut 1993; Cosgrove and Jarvis 2012). Cellulose microfibrils are β-1,4 glucan chains that are held together in parallel by hydrogen bonds and other non-covalent interactions (Jarvis 2003; Nishiyama et al. 2002, 2003). The main hemicellulose in dicotyledonous plants is xyloglucan (XyG), which are β-1,4 glucan chains substituted with xylose (Xyl), galactose (Gal), and fucose (Fuc) sidechains (Fry 1989; Park and Cosgrove 2015). Pectins are galacturonic-acid (GalA) polysaccharides that are mainly found in dicot primary cell walls. Homogalacturonan (HG) are linear α-1,4-galacturonic acid polymers, whereas rhamnogalacturonan-I (RG-I) has an alternating α-1,4-galacturonic acid and rhamnose (Rha) backbone decorated...
These polysaccharides form a three-dimensional structural network that provides not only mechanical stabilities but also extensibility to plant cells. Elucidating the dynamic structures of cell wall polysaccharides and their changes during the plant lifecycle is essential for understanding and controlling plant biochemistry (Cosgrove 2014). While the chemical compositions of plant cell walls have been long known, our knowledge about the packing and interactions of wall polymers has been scarce because of the insoluble and heterogeneous nature of the cell wall. For decades, plant cell wall structural studies have relied on chemical extraction, which disrupts wall integrity, and microscopic imaging, which does not give molecular structural information (Mccann et al. 1990, 1995; Talbott and Ray 1992; Wang and Hong 2015). Bottom-up in vitro binding assays have been used to measure the affinities between different polysaccharides (Lopez-Sanchez et al. 2017; Whitney et al. 1995), but they do not fully recapitulate the molecular interactions in intact native cell walls.

Recently, we demonstrated the application of magic-angle-spinning (MAS) 13C NMR spectroscopy to investigate the structure, dynamics, and intermolecular interactions of intact and native plant cell walls (Dick-Perez et al. 2011; Harris et al. 2012; Wang and Hong 2016; Wang et al. 2014, 2016a). Our approach relies on 13C enrichment of plants and the resolution of 13C chemical shifts of multiple polysaccharides in 2D and 3D 13C–13C correlation NMR spectra. The 13C chemical shift dispersion of carbohydrates is generally smaller than the chemical shift dispersion of proteins because of the similar chemical compositions of sugars. All wall polysaccharides contain 5- or 6-membered sugar rings connected by various glycosidic linkages, and multiple types of linkages can exist for the same sugar. For example, the arabinose rings in RG-I sidechains can be linked at C5, C3, or C2 positions (Phyo et al. 2017a, b; Wang et al. 2014). The 13C chemical shifts of these sugars mostly cluster between 60 and 110 ppm, except for methyl and carboxyl carbons, which resonate outside this range at characteristic chemical shifts of 17 ppm, 54 ppm, and 175 ppm (Dick-Perez et al. 2011, 2012). The 13C linewidths of the polysaccharides depend on the molecular mobility and differ significantly between the rigid cellulose and the dynamic pectins. Cellulose exhibits 13C linewidths of about 2 ppm (Wang et al. 2013) due to the presence of residual 1H–13C dipolar couplings. 13C–13C couplings and conformational heterogeneity, while matrix polysaccharides exhibit narrower 13C linewidths of 0.4–0.8 ppm due to their extensive mobility. These narrow 13C signals are well resolved in 2D J-INADEQUATE spectra, which correlate the sum of the chemical shifts of two bonded carbons with the individual 13C chemical shifts. Using the INADEQUATE experiment, we have resolved the chemical shifts of 7 types of Ara, 7 types of Gal and 10 types of GaLA in Arabidopsis primary cell walls, where the chemical shift multiplicities resulted from the different sugar linkages and conformations (Phyo et al. 2017a, b, 2019). Even for the rigid and chemically simple cellulose, at least 5 types of interior cellulose chemical shifts and 2 types of surface cellulose chemical shifts have been resolved in 2D 13C–13C correlation spectra, indicating the polymorphic conformations of cellulose in plant cell walls (Wang et al. 2016b).

Although 13C-based SSNMR approaches have provided rich structural information on plant cell walls, the requirement of enriching plants with 13C to obtain these 2D and 3D correlation spectra limits the applicability of this approach. 13C enrichment requires growing plants either in liquid culture with 13C-labeled glucose or in growth chambers with 13C-labeled CO2 (Dick-Perez et al. 2012; Dupree et al. 2015; Phyo et al. 2017b; White et al. 2014). The reliance on 13C NMR for structure investigation also limits the ability to resolve the polysaccharide signals and the distance upper bound in probing intermolecular contacts. Due to the low gyromagnetic ratio of 13C spin, 13C–13C dipolar couplings for distances longer than ~8 Å are usually too weak to measure (Hong and Schmidt-Rohr 2013). 1H is an excellent nuclear spin to overcome these limitations: 1H is nearly 100% abundant and has fourfold higher gyromagnetic ratio than 13C, thus 1H–1H distances can in principle be measured to well above 1 nm. While 1H spin diffusion can be indirectly incorporated in the CHHC experiment (Lange et al. 2002) for measuring long-range distances, this CHHC experiment suffers from low sensitivity compared to standard 13C spin diffusion experiments. Moreover, direct incorporation of a 1H chemical shift dimension into 2D and 3D correlation experiments should serve to enhance the resolution of the polysaccharide spectra.

High-sensitivity 1H-detected SSNMR techniques have now been demonstrated for proteins under ultrafast MAS rates of 100 kHz (Andreas et al. 2010; Stanek et al. 2016), where the 1H–1H dipolar couplings of rigid biomolecules are sufficiently averaged to give well resolved spectra. Recently, 1H-detected experiments under 40–100 kHz MAS have also been demonstrated on peptidoglycans of bacterial cell walls (Bougault et al. 2019; Schanda et al. 2014). Under fast MAS, 1H chemical shifts of the glycan backbone and peptide stems were resolved and inter-residue 1H–1H cross peaks were detected using radiofrequency-driven dipolar recoupling (RFDR) (Bennett et al. 1998). In plant cell walls, the matrix polysaccharides are already highly dynamic, thus their 1H linewidths should be narrowed already at MAS frequencies of 50–60 kHz, which can be achieved using 1.3 mm MAS rotors. In comparison, 50–60 kHz MAS is not sufficient for narrowing the 1H signals of the rigid cellulose. Therefore,
$^1$H-detected plant cell wall spectra at 50–60 kHz MAS should selectively detect matrix polysaccharide signals while filtering out cellulose signals, thus simplifying the spectra.

In this study, we demonstrate several $^1$H-incorporated 2D and 3D correlation NMR experiments under MAS frequencies of 10–50 kHz. Both $^{13}$C-detected $^1$H–$^{13}$C–$^{13}$C 3D correlation and $^1$H-detected $^{13}$C–$^1$H 2D correlation experiments are conducted to resolve and assign the $^1$H chemical shifts of matrix polysaccharides. We implement these experiments on intact Arabidopsis primary cell walls, and find good agreement between the $^1$H chemical shifts of the wall polysaccharides and solution-state $^1$H chemical shifts of oligosaccharides obtained from a variety of sources (D’Auria et al. 1992; Habibi et al. 2004; Ishii et al. 2002; Tan et al. 2010; Wu and Mort 2014). We then use these $^1$H chemical shifts to analyze 2D $^1$H–$^{13}$C correlation spectra measured using $^1$H spin diffusion and recoupled $^1$H–$^1$H dipolar interactions. We observed matrix–cellulose cross peaks with short $^1$H mixing times of less than 3 ms, indicating that long-range intermolecular interactions can be observed more efficiently with $^1$H transfer than with $^{13}$C polarization transfer methods.

Materials and methods

Preparation of uniformly $^{13}$C-labeled Arabidopsis primary cell walls

Arabidopsis thaliana primary cell walls were prepared and $^{13}$C-labeled as described before (Dick-Perez et al. 2011; Phyo et al. 2019). Briefly, seedlings were grown in the dark to analyze 2D $^1$H–$^{13}$C correlation spectra measured using $^1$H Larmor frequencies of 10–50 kHz. Both $^{13}$C-detected $^1$H–$^{13}$C–$^{13}$C 3D correlation and $^1$H-detected $^{13}$C–$^1$H 2D correlation experiments are conducted to resolve and assign the $^1$H chemical shifts of matrix polysaccharides. We implement these experiments on intact Arabidopsis primary cell walls, and find good agreement between the $^1$H chemical shifts of the wall polysaccharides and solution-state $^1$H chemical shifts of oligosaccharides obtained from a variety of sources (D’Auria et al. 1992; Habibi et al. 2004; Ishii et al. 2002; Tan et al. 2010; Wu and Mort 2014). We then use these $^1$H chemical shifts to analyze 2D $^1$H–$^{13}$C correlation spectra measured using $^1$H spin diffusion and recoupled $^1$H–$^1$H dipolar interactions. We observed matrix–cellulose cross peaks with short $^1$H mixing times of less than 3 ms, indicating that long-range intermolecular interactions can be observed more efficiently with $^1$H transfer than with $^{13}$C polarization transfer methods.

Solid-state NMR spectroscopy

Solid-state NMR spectra were measured on Bruker Avance II spectrometers at 800 MHz (18.8 Tesla), 600 MHz (14.1 Tesla) and 400 MHz (9.4 Tesla) $^1$H Larmor frequencies. Samples were spun at 12–50 kHz using 4 mm, 3.2 mm, 1.9 mm, and 1.3 mm MAS rotors. Radiofrequency (rf) field strengths were 50–71 kHz for $^{13}$C hard pulses and 60–100 kHz for $^1$H hard pulses and high-power decoupling. For WALTZ decoupling pulses, the rf field strength was typically 10 kHz. All $^{13}$C chemical shifts were externally referenced to the adamantane CH$_2$ peak at 38.48 ppm on the tetramethylsilane (TMS) scale and $^1$H chemical shift were externally reference to the choline headgroup $^1$H signal of POPC lipids at 3.26 ppm on the TMS scale.

A 2D $^1$H-detected refocused $^{13}$C–$^1$H Inverse Nuclear Overhauser Effect (INEPT) experiment (Elena et al. 2005) (Fig. 1a) was conducted under 50 kHz MAS using a 1.3 mm probe. Before and after $^{13}$C t$_1$ evolution, the $^1$H–$^{13}$C INEPT polarization transfer period was 6 ms, and consisted of two delays of $\tau = 1.8$ ms followed by two delays of $\tau' = 1.2$ ms. These values correspond to 1/(4J$_{CH}$) and 1/(6J$_{CH}$), respectively, based on the $^{13}$C–$^1$H J-coupling of 140 Hz (Phyo et al. 2017a; Yu et al. 2012). The 2D INEPT spectrum was measured on D$_2$O-exchanged Arabidopsis primary cell walls. The experiments were conducted at a set temperature of 250 K; at 50 kHz MAS this corresponds to a sample temperature of about 300 K, as estimated from the water $^1$H chemical shift. A 200 ms MISISISIPPI (Zhou and Rienstra 2008) sequence was used to suppress the residual water signal prior to $^1$H detection. The spectral widths were 146.2 ppm (29.4 kHz) for the indirect $^{13}$C dimension and 14.9 ppm (11.9 kHz) for the direct $^1$H dimension. A total of 400 $^{13}$C t$_1$ increments were measured, with a maximum evolution time of 6.8 ms. Per t$_1$ slice 192 scans were averaged, giving a total experimental time of 49 h. WALTZ-16 $^1$H decoupling was applied during the $^{13}$C evolution period, and similarly WALTZ-16 $^{13}$C decoupling was applied during $^1$H detection.

A 2D $^{13}$C–$^{13}$C TOCSY (TOtal Correlated SpectroscopY) spectrum (Bax et al. 1990) was measured to detect multiple-bond $^{13}$C–$^{13}$C correlation peaks within each monosaccharide. The experiments were conducted using a 4 mm probe at 298 K under 12.5 kHz MAS on a 400 MHz spectrometer. A 15 ms $^{13}$C WALTZ-16 sequence with an rf field of 25 kHz was used for $^{13}$C–$^{13}$C isotropic mixing. The spectral widths were 166 ppm (16.6 kHz) for the indirect dimension and 498 ppm (50 kHz) for the direct $^{13}$C dimension. A total of 250 $^{13}$C t$_1$ increments were measured to a maximum evolution time of 7.5 ms. Per t$_1$ slice 64 scans were averaged, giving a total experimental time of 8 h. TPPM $^1$H decoupling was applied at a field strength of 42 kHz during $^{13}$C evolution and detection, and 62 kHz during the $^{13}$C TOCSY mixing period.

3D $^1$H–$^{13}$C–$^{13}$C INEPT-TOCSY spectra (Stanek et al. 2016) were measured under 37 kHz MAS using a 1.9 mm probe to further resolve the $^1$H chemical shifts (Phyo et al. 2017a, b). The set temperature was 273 K, which...
corresponded to a sample temperature of about 298 K. The experiment (Fig. 1b) used INEPT (Elena et al. 2005) for 1H–13C magnetization transfer and TOCSY for 13C–13C magnetization transfer (Bax et al. 1990). The TOCSY mixing period was 11.2 ms, and was sandwiched by a 2 ms z-filter before and after the mixing period to suppress unwanted transverse magnetization. 30 kHz 13C WALTZ-16 isotropic mixing was applied during the TOCSY period. The spectral widths were 5.2 ppm (3.1 kHz) for the 1H F1 dimension, 103 ppm (15.6 kHz) for the 13C F2 dimension, and 414 ppm (62.5 kHz) for the 13C F3 dimension. A total of 54 1H t1 increments and 130 13C t2 increments were acquired, with maximum t1 and t2 evolution times of 8.6 ms and 4.2 ms, respectively. The total experiment time was 54 h. Low-power (10 kHz) 13C and 1H WALTZ-16 decoupling was applied during t1, t2, and t3 periods, while high-power (100 kHz) 1H TPPM decoupling was applied during the 13C TOCSY mixing period.

We also carried out a 2D 1H–13C HETCOR experiment with 1H spin diffusion (Fig. 1c) to measure intermolecular interactions. An initial 1H T2 relaxation filter was used to remove the signals of rigid cellulose before 1H t1 evolution. The 1H chemical shifts of the matrix polysaccharides are then correlated with 13C chemical shifts of the more rigid polysaccharides in the direct dimension. D 2D hChH RFDR experiment to measure long-range interactions between the rigid and dynamic polysaccharides. A short 1H–13C CP period preferentially selects the rigid polysaccharide signals for 13C t1 evolution. The 13C magnetization is transferred to its directly bonded 1H by a 13C–1H reverse CP step, then allowed to couple to other protons by a 1H–1H radiofrequency-driven dipolar recoupling (RFDR) period. The 1H signals of the mobile polysaccharides are then detected after a Hahn echo. The experiment correlates the 13C chemical shifts of rigid polysaccharides with the 1H chemical shifts of dynamic polysaccharides.

Fig. 1 Pulse sequences for measuring 1H chemical shifts of polysaccharides in intact plant cell walls. a 2D 13C–1H INEPT experiment (Elena et al. 2005). b 3D HCC INEPT-TOCSY experiment (Agarwal and Reif 2008; Baldus and Meier 1996; Bax et al. 1990; Hardy et al. 2001; Stanek et al. 2016) for correlating 1H chemical shifts with two 13C chemical shift dimensions. c 2D 1H–13C HETCOR experiment with 1H spin diffusion to measure long-range polysaccharide interactions. An initial 1H T2 relaxation filter was used to remove the signals of rigid cellulose before 1H t1 evolution. The 1H chemical shifts of the matrix polysaccharides are then correlated with 13C chemical shifts of the more rigid polysaccharides in the direct dimension. d 2D hChH RFDR experiment to measure long-range interactions between the rigid and dynamic polysaccharides. A short 1H–13C CP period preferentially selects the rigid polysaccharide signals for 13C t1 evolution. The 13C magnetization is transferred to its directly bonded 1H by a 13C–1H reverse CP step, then allowed to couple to other protons by a 1H–1H radiofrequency-driven dipolar recoupling (RFDR) period. The 1H signals of the mobile polysaccharides are then detected after a Hahn echo. The experiment correlates the 13C chemical shifts of rigid polysaccharides with the 1H chemical shifts of dynamic polysaccharides.
contacts between the matrix polysaccharides and cellulose (Kumashiro et al. 1998; White et al. 2014). The experiment was designed to exploit the different $^1$H $T_2$ relaxation times between mobile and rigid polysaccharides by using a $^1$H $T_2$ filter of 440 μs to suppress the $^1$H magnetization of the rigid cellulose before $^1$H $t_1$ evolution. After $^1$H chemical shift encoding, a 3 ms $^1$H spin diffusion period was used to transfer the magnetization from mobile polysaccharides to cellulose, after which a 100 μs $^1$H–$^{13}$C cross-polarization (CP) step was used for $^{13}$C detection. The spectral widths were 20.7 ppm (16.6 kHz) for the indirect $^1$H dimension and 333 ppm (67 kHz) for the direct $^{13}$C dimension. A total of 120 $^{13}$C $t_1$ increments were measured, giving a maximum evolution time of 3.6 ms, and the number of scans per $t_1$ slice was 448. The total experimental time was 27 h. High-power (62.5 kHz) $^1$H TPPM decoupling was applied during $^{13}$C detection and $^1$H FSLG homonuclear decoupling (Bielecki et al. 1989) at a transverse field of 62.5 kHz was applied during $^1$H $t_1$ evolution. The $^1$H–$^{13}$C HETCOR spectrum was measured under 14.57 kHz MAS at 290 K using a 3.2 mm MAS probe.

Complementing the HETCOR experiment, we conducted a reverse $^1$H-detected hChH experiment (Fig. 1d) (Bougault et al. 2019) to correlate the $^{13}$C chemical shifts of rigid polysaccharides in the indirect dimension with the $^1$H chemical shifts of mobile polysaccharides in the direct dimension. Two short (50 μs) $^1$H–$^{13}$C CP steps were used to select the rigid cellulose signals. A $^1$H RFDR mixing time of 1.28 ms was used after the second, $^{13}$C–$^1$H, CP step to transfer the $^1$H polarization. A $^1$H Hahn echo period of 1.64 ms was applied before detection to suppress the rigid cellulose signals while selectively detecting the dynamic matrix polysaccharide signals. The spectral widths were 97 ppm (14.7 kHz) for the $^{13}$C dimension and 41 ppm (25.0 kHz) for the $^1$H dimension, and the maximum $^{13}$C $t_1$ evolution time was 3.1 ms with 90 $t_1$ increments and 1376 scans per $t_1$ slice. The total experimental time was 62 h. The spectrum was measured under 50 kHz MAS using a 1.3 mm MAS probe.

Results and discussion

Polysaccharides $^1$H chemical shifts from 2D and 3D correlation spectra

We measured the $^1$H chemical shifts of matrix polysaccharides through correlation with the $^{13}$C chemical shifts using the through-bond INEPT polarization transfer sequence. The $^{13}$C chemical shifts of Arabidopsis cell wall matrix polysaccharides have been previously assigned using 2D double-quantum J-INADEQUATE spectra such as that shown in Fig. 2a, which exhibit narrow $^{13}$C linewidths of 0.4–0.8 ppm (Phyo et al. 2017a, b, 2019). To enhance the $^1$H spectral resolution and sensitivity, we spun the cell wall sample at 50 kHz, which is much larger than the $^{13}$C–$^1$H dipolar couplings of the matrix polysaccharides, whose $C$–$H$ order parameters range from 0.3 to 0.6 (Phyo et al. 2017b, 2019; Wang et al. 2016a). The 2D $^{13}$C–$^1$H correlation spectrum (Fig. 2b) was measured without $^1$H–$^1$H homonuclear decoupling during the polarization transfer period, thus the signals of the rigid cellulose, which experience strong $^{13}$C–$^1$H and $^1$H–$^1$H dipolar couplings, are suppressed (Elena et al. 2005). Most of the observed $^1$H chemical shifts fall within the range of 3.5–5.5 ppm (Table 1), with the exception of methyl $^1$H signals, which resonate between 1.0 and 2.0 ppm. 1D $^1$H and $^{13}$C cross sections extracted from the 2D INEPT spectrum (Fig. 2c) indicate $^1$H linewidths of ~0.06 ppm and $^{13}$C linewidths of ~0.7 ppm for the matrix polysaccharides. Fast MAS narrowed the linewidths and increased the sensitivities of the spectra compared to moderate MAS. The 1D $^{13}$C INEPT spectra measured under 50 kHz gave twofold higher intensities than spectra measured at 10 kHz MAS (Phyo et al. 2017b) for the pectin backbone GalA and the Xyl-sidechain Xyl signals (Fig. 2d). In comparison, the signals of the highly dynamic Ara in RG-I sidechains do not differ significantly between the two spinning speeds, as expected.

Based on the known $^{13}$C chemical shifts of Arabidopsis seedling cell walls, we assigned many $^1$H chemical shifts of Ara, Gal, GalA and Rha in the 2D INEPT spectrum (Table 1). A weak cross peak at (102.4, 4.40) ppm is noteworthy. This 102-ppm $^{13}$C chemical shift had not been assigned in previous 2D J-INADEQUATE spectra of Arabidopsis primary wall samples (Dick-Perez et al. 2012; Phyo et al. 2017a, b, 2019; Wang et al. 2015). However, this $^{13}$C chemical shift was clearly detected and resolved in Arabidopsis secondary cell walls and in Brachypodium grass primary cell walls (Simmons et al. 2016; Wang et al. 2014), where it was assigned to xylan (Xn) C1. Solution NMR studies of oligosaccharides from plant, microbial and fungal cell walls indicate that a 102-ppm $^{13}$C chemical shift can in principle result from mannose (Man) C1 (Kobayashi et al. 1995, 1997), terminal (t) reducing Ara in RG-I sidechains do not differ significantly between the two spinning speeds, as expected.
et al. 2013) but absent in dicot primary cell walls. The only sugar whose 102-ppm 13C chemical shift has a compatible H1 chemical shift is Xn C1, whose 1H chemical shift falls within 4.41–4.55 ppm (Pena et al. 2016). Enzymes such as IRX9L, IRX10L and IRX14 have been shown to synthesize xylan in Arabidopsis primary cell walls; this xylan has C1–H1 chemical shifts of ~102 ppm and ~4.5 ppm, respectively (Mortimer et al. 2015). Based on this literature evidence, we assign the (102.4, 4.40) ppm cross peak to Xn C1–H1, even though additional correlation peaks are difficult to resolve in the 2D INEPT spectrum.

The presence of a Xn C1–H1 cross peak in the INEPT spectrum motivated us to re-examine the 2D 13C–13C J-INADEQUATE spectrum. Previously two unassigned 13C chemical shifts at 82 ppm and 64 ppm were reported in the 2D J-INADEQUATE spectrum of Arabidopsis primary cell wall (Dick-Perez et al. 2012). Subsequently, these two 13C chemical shifts were observed in Arabidopsis

Fig. 2 1H and 13C chemical shift assignments of Arabidopsis seedling cell walls measured on an 800-MHz spectrometer. a 2D J-INADEQUATE spectrum measured under 12 kHz MAS (Phyo et al. 2019). Superscripts denote different subtypes of each sugar ring. Boxed regions at double-quantum 13C chemical shifts of 160 and 170 ppm show the GaLA C6 carbonyl signals and Rha-C6 signals, respectively. b 2D CH INEPT spectrum measured under 50 kHz MAS. Superscripts denote different subtypes of each monosaccharides. Ambiguous assignments are shown in orange. c Selected 1H and 13C cross sections of the 2D INEPT spectrum (red dashed lines). d 1D 13C INEPT spectrum measured under 50 kHz MAS (red) shows higher intensities than spectrum measured at 10 kHz MAS (black), particularly for the GaLA backbone of pectins and Xyl sidechain of xyloglucan.
secondary cell walls and were assigned to Xn C4 and C5 (Simmons et al. 2016). These signals, although weak, suggest that xylan is present at a low concentration in primary cell walls. With this identification, we can also tentatively assign a (80.9, 3.67) ppm cross peak in the INEPT spectrum (Fig. 2b) to Xn C4–H4.

The 2D $^{13}$C–$^1$H INEPT spectrum does not fully resolve all cross peaks, especially in the congested $^{13}$C chemical shift range of 70–80 ppm. For example, Rha C4, GalA C3/ C5, Gal C2 and Xyl C2 signals all resonate at about 72 ppm, (Phyo et al. 2017a, b, Phyo et al. 2019), thus their directly bonded $^1$H chemical shifts are not completely resolved. This motivated us to measure a 3D HCC spectrum where two $^{13}$C chemical shift dimensions are used to resolve the $^1$H chemical shifts. Figure 3 shows a 2D $^{13}$C–$^1$H TOCSY spectrum measured with a 15 ms mixing time under 12.5 kHz MAS. At this moderate MAS speed, the 2D spectrum mainly exhibits the Ara and Gal signals of RG-I sidechains. With faster MAS, we expect more polysaccharide signals to appear. Figure 4 shows the 3D HCC INEPT-TOCSY spectrum (Agarwal and Reif 2008; Hardy et al. 2001) and representative $^{13}$C–$^1$C 2D cross sections.

| Sugar | Linkage | H1 (ppm) | H2 (ppm) | H3 (ppm) | H4 (ppm) | H5 (ppm) | H6 (ppm) | CH$_3$ (ppm) | OCH$_3$(ppm) |
|-------|---------|----------|----------|----------|----------|----------|----------|-------------|-------------|
| Ara$^a$| 2,5-Ara | 5.14 | 4.11 | – | – | – | – | – | – |
|       |         | 107.1 | 87.7 | – | – | – | – | – | – |
| Ara$^b$| 2,3,5-Ara | 5.18 | 4.25 | – | – | – | – | – | – |
|       |         | 107.0 | 85.8 | – | – | – | – | – | – |
| Ara$^c$| 5-Ara | 5.04 | 4.10 | 3.96 | 4.15 | 3.80 | – | – | – |
|       |         | 108.2 | 81.8 | 77.7 | 83.1 | 67.6 | – | – | – |
| Ara$^d$| t-Ara | 5.06 | 4.10 | 3.93 | 4.01 | 3.72 | – | – | – |
|       |         | 107.7 | 82.3 | 77.4 | 84.5 | 62.0 | – | – | – |
| Ara$^e$| – | – | 4.23 | – | – | – | – | – | – |
|       |         | 108.2 | 80.1 | – | – | – | – | – | – |
| Ara$^f$| 2,5-Ara | 5.30 | 4.18 | – | – | – | – | – | – |
|       |         | 108.5 | 88.0 | – | – | – | – | – | – |
| Ara$^h$| 5-Ara | 5.14 | 4.11 | 3.94 | – | – | – | – | – |
|       |         | 109.9 | 82.0 | 77.5 | – | – | – | – | – |
| Gal$^a$| 4-Gal | 5.12 | 3.88 | 4.34 | – | – | – | – | – |
|       |         | 109.8 | 69.0 | 69.7 | 78.5 | 72.1 | – | – | – |
| Gal$^b$| t-Gal | 5.09 | 3.72 | – | – | – | – | – | – |
|       |         | 101.0 | 68.6 | 71.3 | – | – | – | – | – |
| Gal$^c$| – | – | 3.88 | 4.34 | – | – | – | – | – |
|       |         | 104.1 | 71.4 | – | – | – | – | – | – |
| GalA$^a$| 4-GalA | 4.58 | 3.64 | 3.72 | 4.11 | 3.66 | 3.75 | – | – |
|       |         | 105.1 | 72.8 | 74.0 | 78.4 | 75.2 | 61.5 | – | – |
| GalA$^b$| – | – | 3.61 | 3.90 | 3.65 | 3.73 | – | – | – |
|       |         | 105.2 | 72.0 | 73.4 | 69.5 | 75.6 | 61.7 | – | – |
| GalA$^c$| – | – | 3.72 | 3.88 | 4.34 | – | – | – | – |
|       |         | 98.8 | 67.8 | 72.4 | 77.8 | 71.2 | 171.5 | 53.7 | – |
| GalA$^d$| – | – | 3.72 | 3.88 | 4.34 | – | – | – | – |
|       |         | 98.2 | 68.7 | 69.7 | 78.5 | 72.1 | – | – | – |
| Rha$^b$| 2-Rha | 5.13 | 4.31, 4.39 | 3.55 | 4.01 | 3.72 | 3.94 | 1.26 | 2.03, 2.11 |
|       |         | 101.2 | 79.8 | 71.0 | 72.6 | 68.1 | 16.0 | 20.0, 21.3 | – |

Ambiguous $^1$H chemical shifts are italicized.

Table 1 $^1$H chemical shifts measured using the 2D CH INEPT and 3D HCC experiments, together with previously assigned $^{13}$C chemical shifts (Phyo et al. 2017a, b, 2019) of matrix polysaccharides.
found $^1H$ linewidths of 0.22 ppm for the $F_1$ dimension, $^{13}C$ linewidths of 1.2 ppm for the indirect $F_2$ dimension, and $^{13}C$ linewidths of about 0.5 ppm for the direct dimension. The 3D spectrum allows the assignment of 5-Ara, t-Ara, 4-Gal and t-Gal $^1H$ chemical shifts (Fig. 5, Table 1). The 4-Gal and t-Gal sugars display similar $^1H$ chemical shifts except for H4 and H3: the H4 chemical shift is 4.11 ppm for 4-Gal and 3.90 ppm for t-Gal, consistent with the trend of increasing chemical shift at the linkage position. The H3 chemical shift is 3.72 ppm for 4-Gal and 3.61 ppm for t-Gal, indicating that the H3 chemical shift is also sensitive to the C4 linkage. In Ara, a similar $^1H$ chemical shift increase near the glycosidic linkage: the H4 and H5 chemical shifts are 0.08 and 0.14 ppm larger for 5-Ara than for t-Ara (Table 1). We observed a significant spread in the Ara H1 chemical shifts from 5.04 to 5.30 ppm, which may result from differences in the glycosidic bond angles and interactions of arabinose with other polysaccharides. The H2 chemical shifts of Ara-a, Ara-b and Ara-f showed no clear trend for 2-linked Ara. We detected two sets of Gal H1–C1 chemical shifts, at (5.48, 105) ppm and (4.40–4.43, 103–104) ppm. These chemical shifts are in good agreement with the solution state Gal H1 chemical shift range of 4.50–4.66 ppm. We hypothesize that these chemical shift multiplicities may indicate conformational differences of Gal in RG-I and XyG sidechains.

Under 37 kHz MAS, the pectin backbone Rha and GalA signals are only partially resolved due to the relative immobility of the polysaccharide backbone compared to the sidechains and residual $^{13}C$ chemical shift overlap. For example, the $^{13}C$–$^{13}C$ cross peak at (69.7, 71–72) ppm can be assigned to GalA (C2/C3, C3/C5), Rha (C5, C3/C4), Gal (C4, C2) or Xyl (C4, C2) (Table 1). Thus, some of the pectin backbone signals and Xyl signals are not fully resolved in the 3D spectrum. Due to the higher rigidity of Xyl, we could assign only the H4 signal at 3.62 ppm. Combining the 2D CH and 3D HCC spectra, we assigned 5 of the 6 C–H groups in Rha, 4 of the 5 C–H groups in GaLA, and 1 out of 5 C–H groups in Xyl. Figure 5e compares these Arabidopsis primary wall polysaccharide $^1H$ chemical shifts with solution state $^1H$ chemical shifts of oligosaccharides digested from the cell walls of various organisms such as pear fruit skin (Habibi et al. 2004), apple (D’Auria et al. 1992; Ishii et al. 2002; Tan et al. 2010; Wu and Mort 2014), bamboo shoots and destarched potato cell wall (D’Auria et al. 1992; Ishii et al. 2002; Tan et al. 2010; Wu and Mort 2014), pacific sponge (D’Auria et al. 1992; Ishii et al. 2002; Tan et al. 2010; Wu and Mort 2014), and hydroxyproline-arabinogalactan of glycoproteins from tobacco cells, (D’Auria et al. 1992; Ishii et al. 2002; Tan et al. 2010; Wu and Mort 2014). We found excellent agreement between the solution and solid-state $^1H$ chemical shifts, with a slope of 0.99 and a correlation coefficient of 0.99.

**Cellulose–matrix polysaccharide interactions from 2D $^1H$–$^{13}C$ correlation spectra**

The availability of $^1H$ chemical shifts of matrix polysaccharides allows us to probe intermolecular contacts using the much stronger $^1H$–$^1H$ dipolar couplings rather than the $^{13}C$–$^{13}C$ dipolar couplings. So far most data about cellulose interactions with matrix polysaccharides derive from 2D and 3D $^{13}C$–$^{13}C$ correlation spectra measured using long $^{13}C$ spin diffusion mixing times (Dick-Perez et al. 2012; Phyo et al. 2017a, b, 2019; Wang et al. 2015, 2016a). Due to the fourfold lower gyromagnetic ratio of $^{13}C$ compared to $^1H$ spins, the distance upper limit that is accessible by $^{13}C$ spin diffusion is much shorter than $^1H$ (Hong and Schmidt-Rohr 2013). Thus, it is of interest to explore the use of $^1H$–$^1H$ dipolar couplings to increase the distance reach.

Figure 6 shows the 2D $^1H$–$^{13}C$ HETCOR spectrum of Arabidopsis seedling cell walls measured at 14.57 kHz MAS. We used a $^1H$ T2 filter to suppress the rigid cellulose signals in the indirect $^1H$ dimension. Since the resolved 89-ppm $^{13}C$ signal is exclusively due to interior crystalline cellulose, we optimized the $^1H$ T2 filter time by suppressing the 89-ppm peak as much as possible in the absence of $^1H$ spin diffusion. With a $^1H$ T2 filter of 440 μs, we suppressed the 89-ppm peak to less than ~0.5% of its full intensity.
(Fig. 6a) while the 100-ppm matrix polysaccharide C1 signal is retained to 15% of the full intensity. After $^1$H chemical shift encoding, the $^1$H magnetization of the dynamic matrix polysaccharides is transferred to cellulose using 3 ms $^1$H spin diffusion, and the result of the transfer is detected on $^{13}$C after a $^1$H–$^{13}$C CP period of 100 μs. The experiment
therefore correlates the dynamic polysaccharides' $^1$H chemical shifts with the rigid polysaccharides' $^{13}$C chemical shifts. In addition, the 100 μs CP transfer not only selects for the rigid cellulose signals but also permits partial polarization transfer to moderately dynamic matrix polysaccharides (Dregni et al. 2019), thus we also detected matrix–matrix cross peaks. The 2D HETCOR spectrum (Fig. 6b) displays several weak but unambiguous pectin–cellulose cross peaks. Two cross peaks at (4.45, 88.7) ppm and (4.48, 65.4) ppm can be assigned to the correlation of GalA H4, Gal H1 or Xn H1 with interior cellulose C4 and C6. Although the $^1$H chemical shift of 4.45 ppm is not uniquely assigned, the Gal sidechain of RG-I is less likely than the pectin backbone to interact with cellulose microfibrils due to sidechain conformational disorder (Wang et al. 2012, 2015). In comparison, xylan interactions with cellulose have been observed in secondary cell walls (Simmons et al. 2016); thus, although the xylan amount in the Arabidopsis primary wall is low, it is possible that the cross peaks at 4.40 ppm may result from xylan–cellulose spatial contact. Future experiments will be necessary to unambiguously identify the matrix polysaccharides that contact cellulose microfibrils. We also observed cross peaks at (3.36, 89.1) ppm and (3.36, 65.3) ppm, which we assign to correlations between Gal H2/Rha H4 and interior cellulose C4 and C6. These sparse but important intermolecular $^1$H–$^{13}$C correlations demonstrate that the 2D $^1$H–$^{13}$C correlation experiments, suitably designed to filter out some of the polysaccharide signals based on molecular dynamics, is useful for probing cellulose–pectin interactions in these plant cell walls.

To further explore whether $^1$H detection under fast MAS can increase the sensitivity for detecting intermolecular contacts, we carried out a reverse 2D experiment where $^{13}$C chemical shifts of the rigid polysaccharides are probed in the indirect dimension and are correlated with $^1$H chemical shifts of the dynamic matrix polysaccharides in the direct dimension. We spun the sample at 50 kHz and used $^1$H RFDR to recouple the $^1$H–$^1$H dipolar interaction for polarization transfer. A $^1$H T$_2$ filter was used before detection to suppress the broad cellulose–cellulose cross peaks and retain

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**Fig. 5** Resolved $^1$H and $^{13}$C chemical shifts of matrix polysaccharides in the Arabidopsis primary cell walls. a Galacturonic acid chemical shifts. b Rhamnose chemical shifts in RG-I. c Arabinose chemical shifts. d Galactose chemical shifts. e Correlation between solution NMR $^1$H chemical shifts of oligosaccharides (D’Auria et al. 1992; Habibi et al. 2004; Ishii et al. 2002; Tan et al. 2010; Wu and Mort 2014) and solid-state NMR $^1$H chemical shifts of Arabidopsis wall matrix polysaccharides measured here.

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only cellulose–matrix polysaccharide cross peaks. Figure 7 shows the 2D hChH RFDR spectrum, where a residual water 1H signal at ~ 4.75 ppm and strong signals at 13C chemical shifts of 70–75 ppm are observed. Among the isolated peaks, we detected two weak cross peaks that can be assigned to cellulose–matrix correlations: a (65.4, 3.60) ppm cross peak can be assigned to interior cellulose C6 correlation to Gal H2, Xyl H4, or Ara H5 protons, while a (72.7, 2.00) ppm cross peak can be assigned to cellulose C2/C5 correlation with the acetyl proton in RG-I. Although the 72.7 ppm peak could also result from matrix polysaccharide carbons, the use of a very short 1H–13C CP transfer of 50 μs should preferentially enhance the rigid cellulose 13C signals in the indirect dimension. Additionally, the second 13C–1H CP period of 50 μs should further suppress the dynamic polysaccharide signals. Overall, the sensitivity of this 1H-detected 2D hChH spectrum is slightly lower than the 13C-detected HETCOR.

We compared the signal-to-noise ratios (SNR) of three cross peaks at (104.4, 2.0) ppm, (72.4, 2.0) ppm and (64.1, 2.0) ppm. We divided the peak intensities by the square root of the experimental time and by the static magnetic field B0 to the 3/2 power. The 1H-detected peaks have 0.5–1.0 times the SNRs of the 13C-detected peaks. The 1H-detected experiments conducted on the 1.3 mm MAS probe and the 13C-detected experiments conducted on the 3.2 mm probe both used fully packed rotors, which contained ~ 2 mg and ~ 45 mg material, respectively. Thus, the eightfold gain in sensitivity by 1H detection and the higher filling factor due to the small rotor offset the reduction in sample amount (Lecoq et al. 2019; Mandala and Hong 2019). Moreover, the residual water 1H peak complicates the measurement of matrix polysaccharide 1H signals near 4.75 ppm, which include the H1 of Ara, Gal and GalA. More complete H/D exchange and more efficient water suppression techniques will be important for applying 1H-detected NMR techniques to these hydrated polysaccharide cell walls.
The current data demonstrate the usefulness of $^1$H chemical shifts and $^1$H detection for high-sensitivity studies of the structures and intermolecular packing of complex cell walls. Previous $^1$H-detected $^{13}$C–$^1$H and $^{15}$N–$^1$H correlation experiments at 100 kHz MAS have been applied to peptidoglycans of bacterial cell walls (Bougault et al. 2019). The $^1$H chemical shift assignment and $^1$H RFDR recoupling allowed the observation of long-range correlations between $\text{N}-\text{acetylglucosamine}$ and $\text{N}-\text{acetylmuramic acid}$ in the backbone and between the glycan backbone and the peptide stems. The C–H order parameters of free peptidoglycans were found to be 0.2–0.3 for the peptide and 0.6 for the glycan backbone (Schanda et al. 2014). These values are similar to the order parameters of matrix polysaccharides in plant cell walls (Phyo et al. 2017b, 2019; Wang et al. 2016a). Therefore, the techniques developed in this study for plant primary cell walls should also be useful for studying peptidoglycans in bacterial cell walls under 50–60 kHz MAS.

**Conclusion**

We have shown that $^1$H chemical shifts provide a useful new source of information for plant cell wall studies. These $^1$H-based MAS NMR techniques not only further resolve the polysaccharide signals but also allow more efficient measurement of long-range intermolecular contacts. The $^1$H chemical shifts can be resolved and assigned using either $^1$H-detected 2D and 3D correlation experiments at fast MAS rates of 30–60 kHz or $^{13}$C-detected experiments at moderate MAS rates of 10–20 kHz. For spectral resolution and assignment, through-bond polarization transfer methods such as INEPT and TOCSY are ideal, while for investigating intermolecular contacts, dipolar polarization transfer using $^1$H–$^1$H recoupling is desirable. The incorporation of $^1$H chemical shifts as a new dimension in the 2D and 3D correlation NMR spectra can also reduce the demand for $^{13}$C enrichment in cell wall structural studies (Kirui et al. 2019).

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**References**

Agarwal V, Reif B (2008) Residual methyl protonation in perdeuterated proteins for multi-dimensional correlation experiments in MAS solid-state NMR spectroscopy. J Magn Reson 194:16–24

Andreas LB et al (2016) Structure of fully protonated proteins by proton-detected magic-angle spinning NMR. Proc Natl Acad Sci USA 113:9187–9192

Baldus M, Meier BH (1996) Total correlation spectroscopy in the solid state. The use of scalar couplings to determine the through-bond connectivity. J Magn Reson A 121:65–69

Bax A, Clore GM, Gronenborn AM (1990) $^1$H-1H correlation via isotropic mixing of $^{13}$C magnetization, a new 3-dimensional approach for assigning $^1$H and $^{13}$C spectra of $^{13}$C-enriched proteins. J Magn Reson 88:425–431

Bennett AE, Rienstra CM, Griffiths JM, Zhen WG, Lansbury PT, Grif-fin RG (1998) Homonuclear radio frequency-driven recoupling in rotating solids. J Chem Phys 108:9463–9479

Bielecki A, Kolbert AC, Levitt MH (1989) Frequency-switched pulse sequences—homonuclear decoupling and dilute spin NMR in sol-sids. Chem Phys Lett 155:341–346

Bougault C, Ayala I, Vollmer W, Simorre JP, Schanda P (2019) Studying intact bacterial peptidoglycan by proton-detected NMR spectroscopy at 100 kHz MAS frequency. J Struct Biol 206:66–72

Caffall KH, Mohnen D (2009) The structure, function, and biosynthesis of plant cell wall pectic polysaccharides. Carbohydr Res 344:1879–1900

Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants; consistency of molecular structure with the physical properties of the walls during growth. Plant J 3:1–30

Cosgrove DJ (2014) Re-constructing our models of cellulose and primary cell wall assembly. Curr Opin Plant Biol 22:122–131

Cosgrove DJ, Jarvis MC (2012) Comparative structure and biomechanics of plant primary and secondary cell walls. Front Plant Sci 3:204

D’Auria M, Paloma LG, Minale L, Riccio R (1992) Structure characterization by two-dimensional NMR spectroscopy, of two marine triterpene oligoglycosides from a pacific sponge of the genus *Erylus*. Tetrahedron 48:491–498

Dick-Perez M, Zhang YA, Hayes J, Salazar A, Zabotina OA, Hong M (2011) Structure and interactions of plant cell wall polysaccharides by two- and three-dimensional magic-angle-spinning solid-state NMR. Biochemistry 50:989–1000

Dick-Perez M, Wang T, Salazar A, Zabotina OA, Hong M (2012) Multidimensional solid-state NMR studies of the structure and
dynamics of pectic polysaccharides in uniformly $^{13}$C-labeled Arabidopsis primary cell walls. Magn Reson Chem 50:539–550
Dregni AJ et al (2019) In vitro ON4R tau fibrils contain a monomorphic b-sheet core enclosed by dynamically heterogeneous fuzzy coat segments. Proc Natl Acad Sci USA 116:16357–16366
Dupree R, Simmons TJ, Mortimer JC, Patel D, Iuga D, Brown SP, Dupree P (2015) Probing the molecular architecture of Arabidopsis thaliana secondary cell walls using two- and three-dimensional $^{13}$C solid state nuclear magnetic resonance spectroscopy. Biochemistry 54:2335–2345
Elena B, Lesage A, Steuer Nagel S, Bockmann A, Emsley L (2005) Proton to carbon-13 INEPT in solid-state NMR spectroscopy. J Am Chem Soc 127:17296–17302
Fry SC (1989) The structure and functions of xyloglucan. J Exp Bot 40:1–11
Habibi Y, Heyraud A, Mahrouz M, Vignon MR (2004) Structural features of pectic polysaccharides from the skin of Opuntia ficus-indica prickly pear fruits. Carbohydr Res 339:1119–1127
Hardy EH, Verel R, Meier BH (2001) Fast MAS total through-bond correlation spectroscopy. J Magn Reson 148:459–464
Harris DM et al (2012) Cellulose microfibril crystallinity is reduced by mutating C-terminal transmembrane region residues CESAl503V and CESAz523 of cellulose synthase. Proc Natl Acad Sci USA 109:4098–4103
Hong M, Schmidt-Rohr K (2013) Magic-angle-spinning NMR techniques for measuring long-range distances in biological macromolecules. Acc Chem Res 46:2154–2163
Ishii T, Ichita J, Matsue H, Ono H, Maeda I (2002) Fluorescent labeling of pectic oligosaccharides with 2-aminobenzamide and enzyme assay for pectin. Carbohydr Res 337:1023–1032
Jarvis M (2003) Chemistry: cellulose stacks up. Nature 426:611–612
Kirui A, Ling Z, Kang X, Dickwella Widanage MC, Mentink-Vigier M, Lecoq L et al (2019) 100 kHz MAS proton-detected NMR spectroscopy of cellulose β-1,4-galactan from G. hirsutum. J Magn Reson 328:329–338
Kobayashi H et al (1995) Assignment of $^1$H and $^{13}$C NMR chemical-shifts of D-mannan composed of α-(1→2)-linkage and α-(1→6)-linkage obtained from Candida kefyr IFO 0586 strain. Carbohydr Res 267:299–306
Kobayashi H et al (1997) Structure of a cell wall mannan from the pathogenic yeast, Candida catenulata: assignment of $^1$H nuclear magnetic resonance chemical shifts of the inner α-1,6-linked mannan residues substituted by a side chain. Arch Biochem Biophys 341:70–74
Kumashiro KK, Schmidt-Rohr K, Murphy OJ, Ouellette KL, Cramer LS, Luca S, Baldus M (2002) Structural constraints from proton-nucleus detection. J Am Chem Soc 120:5043–5051
Laguri C et al (2018) Solid state NMR studies of intact lipopolysaccharide endotoxin. ACS Chem Biol 13:2106–2113
Lange A, Luca S, Baldus M (2002) Structural constraints from proton-mediated rare-spin correlation spectroscopy in rotating solids. J Am Chem Soc 124:9704–9705
LeCoq L et al (2019) 100 kHz MAS proton-detected NMR spectroscopy of hepatitis B virus capsids. Front Mol Biosci 6:58
Lopez-Sanchez P, Martinez-Sanz M, Bonilla MR, Wang D, Gilbert EP, Stokes JR, Sidley MJ (2017) Cellulose-pectin composite hydrogels: intermolecular interactions and material properties depend on order of assembly. Carbohydr Polym 162:71–81
Lowman DW et al (2011) New insights into the structure of (1→3,1→6)-β-D-glucan side chains in the Candida glabrata cell wall. PLoS ONE 6:e27614
Mandala VS, Hong M (2019) High-sensitivity protein solid-state NMR spectroscopy. Curr Opin Struct Biol. https://doi.org/10.1016/j.csi.2019.03.027
Mccann MC, Wells B, Roberts K (1990) Direct visualization of cross-links in the primary plant cell wall. J Cell Sci 96:323–334
Mccann MC, Roberts K, Wilson RH, Sidley MJ, Gibeaut DM, Kim JB, Carpita NC (1995) Old and new ways to probe plant cell wall architecture. Can J Bot 73:S103–S113
Mortimer JC et al (2015) An unusual xylan in Arabidopsis primary cell walls is synthesised by GXU3, IRX9L, IRX10L and IRX14. Plant J 83:413–426
Nars A et al (2013) Aphanomyces euteiches cell wall fractions containing novel glucan-chitosaccharides induce defense genes and nuclear calcium oscillations in the plant host Medicago truncatula. PLoS ONE 8:e75039
Nishiyama Y, Langan P, Chancy H (2002) Crystal structure and hydrogen-bonding system in cellulose Iα, from synchrotron X-ray and neutron fiber diffraction. J Am Chem Soc 124:9074–9082
Nishiyama Y, Sugiyama Y, Chancy H, Langan P (2003) Crystal structure and hydrogen bonding system in cellulose Iα, from synchrotron X-ray and neutron fiber diffraction. J Am Chem Soc 125:14300–14306
Park YB, Cosgrove DJ (2015) Xyloglucan and its interactions with other components of the growing cell wall. Plant Cell Physiol 56:180–194
Pena MJ, Kulkarni AR, Backe J, Boyd M, O’Neill MA, York WS (2016) Structural diversity of xylans in the cell walls of monocots. Planta 244:589–606
Phyo P, Wang T, Kiemle SN, O’Neill H, Pingali SV, Hong M, Cosgrove DJ (2017a) Gradients in wall mechanics and polysaccharides along growing inflorescence stems. Plant Physiol 175:1593–1607
Phyo P, Wang T, Xiao C, Anderson CT, Hong M (2017b) Effects of pectin molecular weight changes on the structure, dynamics, and polysaccharide interactions of primary cell walls of Arabidopsis thaliana: insights from solid-state NMR. Biomacromol 18:2937–2950
Phyo P, Gu Y, Hong M (2019) Impact of acidic pH on plant cell wall polysaccharide structure and dynamics: insights into the mechanism of acid growth in plants from solid-state NMR. Cellulose 26:291–304
Schanda P et al (2014) Atomic model of a cell-wall cross-linking enzyme in complex with an intact bacterial peptidoglycan. J Am Chem Soc 136:17852–17860
Simmons TJ et al (2016) Folding of xylan onto cellulose fibrils in plant cell walls revealed by solid-state NMR. Nat Commun 7:13902
Stanek J et al (2016) NMR spectroscopic assignment of backbone and side-chain protons in fully protonated proteins: microcrystals, sedimented assemblies, and amyloid fibrils. Angew Chem Int Ed Engl 55:15504–15509
Talbott LD, Ray PM (1992) Molecular-size and separability features of Pea cell wall polysaccharides. Implications for models of primary wall structure. Plant Physiol 98:357–368
Tan L, Varnai P, Lamport DT, Yuan C, Xu J, Qiu F, Kieliszewski MJ (2010) Plant O-hydroxyproline arabinogalactans are composed of repeating trigalactosyl subunits with short bifurcated side chains. J Biol Chem 285:24575–24583
Wang T, Hong M (2015) Solid-state NMR investigations of cellulose structure and interactions with matrix polysaccharides in plant primary cell walls. J Exp Bot 66:503–514
Wang T, Hong M (2016) Solid-state NMR investigations of cellulose structure and interactions with matrix polysaccharides in plant primary cell walls. J Exp Bot 67:503–514
Wang T, Zabotina O, Hong M (2012) Pectin-cellulose interactions in the Arabidopsis primary cell wall from two-dimensional magic-angle-spinning solid-state nuclear magnetic resonance. Biochimie 51:9846–9856
Wang T, Park YB, Caporini MA, Rosay M, Zhong LH, Cosgrove DJ, Hong M (2013) Sensitivity-enhanced solid-state NMR detection
of expansin’s target in plant cell walls. Proc Natl Acad Sci USA 110:16444–16449
Wang T, Salazar A, Zabotina OA, Hong M (2014) Structure and dynamics of Brachypodium primary cell wall polysaccharides from two-dimensional $^{13}$C solid-state nuclear magnetic resonance spectroscopy. Biochemistry 53:2840–2854
Wang T, Park YB, Cosgrove DJ, Hong M (2015) Cellulose-pectin spatial contacts are inherent to never-dried Arabidopsis thaliana primary cell walls: evidence from solid-state NMR. Plant Physiol 168:871–883
Wang T, Phyo P, Hong M (2016a) Multidimensional solid-state NMR spectroscopy of plant cell walls. Solid State Nucl Magn Reson 78:56–63
Wang T, Yang H, Kubicki JD, Hong M (2016b) Cellulose structural polymorphism in plant primary cell walls investigated by high-field 2D solid-state NMR spectroscopy and density functional theory calculations. Biomacromolecules 17:2210–2222
Wefers D, Tyl CE, Bunzel M (2014) Novel arabinan and galactan oligosaccharides from dicotyledonous plants. Front Chem 2:100
White PB, Wang T, Park YB, Cosgrove DJ, Hong M (2014) Water-polysaccharide interactions in the primary cell wall of Arabidopsis thaliana from polarization transfer solid-state NMR. J Am Chem Soc 136:10399–10409
Whitney SEC, Brigham JE, Darke AH, Reid JSG, Gidley MJ (1995) In vitro assembly of cellulose/xyloglucan networks—ultrastructural and molecular aspects. Plant J 8:491–504
Wu X, Mort A (2014) Structure of a rhamnogalacturonan fragment from apple pectin: implications for pectin architecture. Int J Carbohydr Chem 2014:6
Yu B, van Ingen H, Vivekanandan S, Rademacher C, Norris SE, Freedberg DI (2012) More accurate 1 J(CH) coupling measurement in the presence of 3 J(HH) strong coupling in natural abundance. J Magn Reson 215:10–22
Zhou DH, Rienstra CM (2008) High-performance solvent suppression for proton detected solid-state NMR. J Magn Reson 192:167–172

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