Supplementary Information

Comparative Analysis of Polysaccharide and Cell Wall Structure in *Aspergillus nidulans* and *Aspergillus fumigatus* by Solid-State NMR

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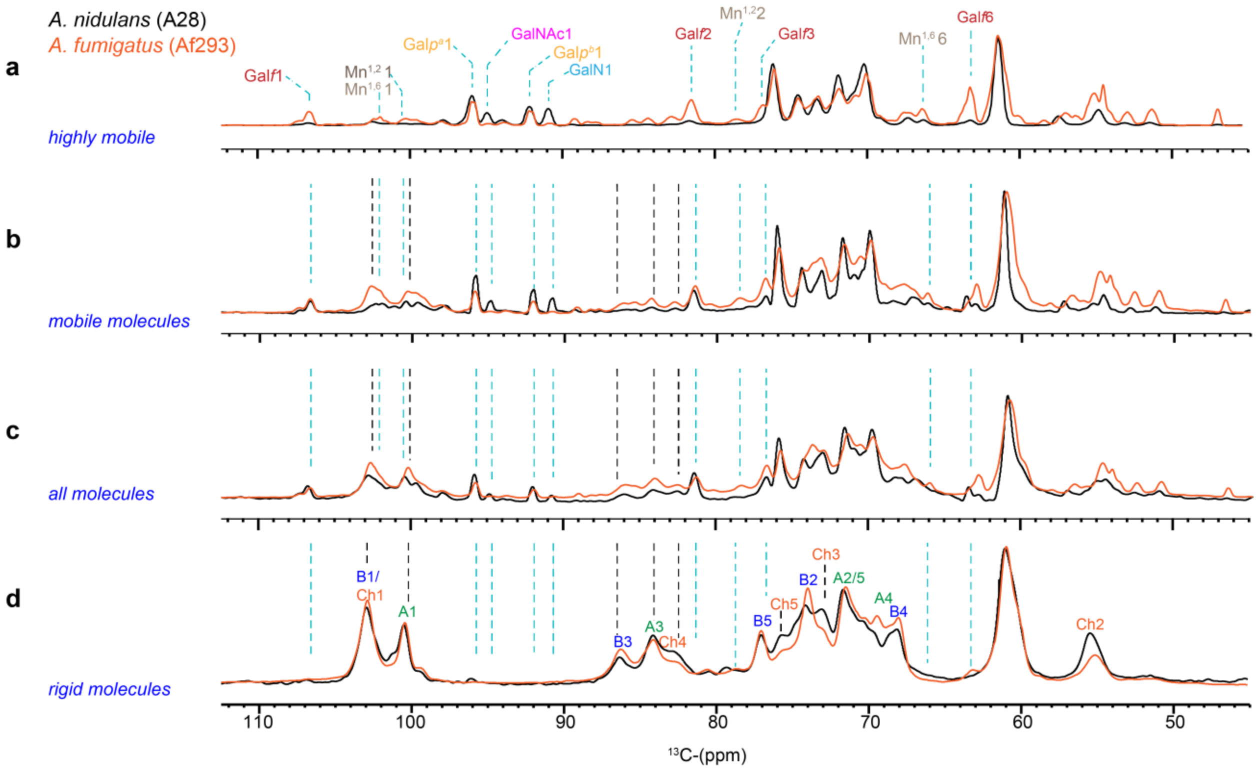
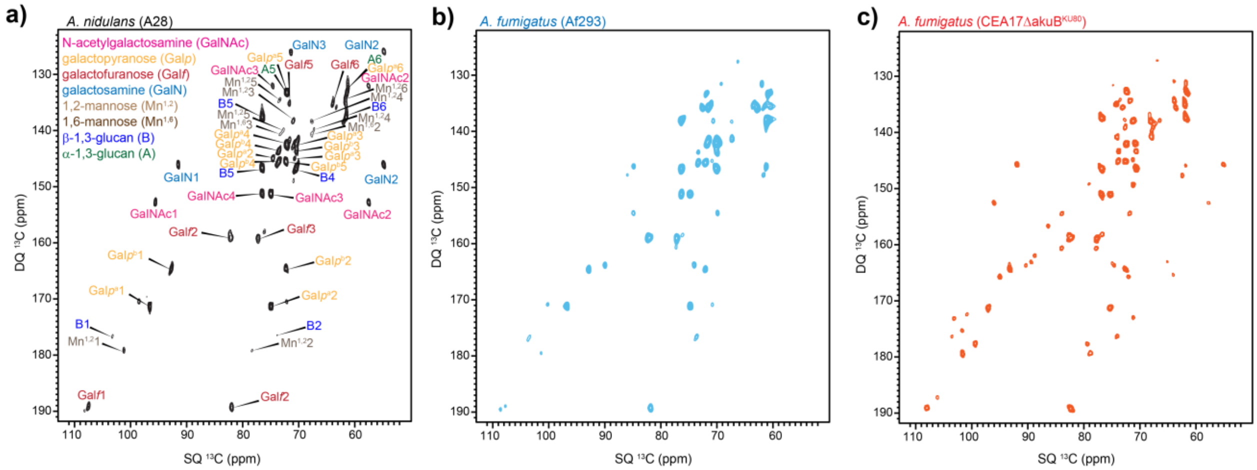
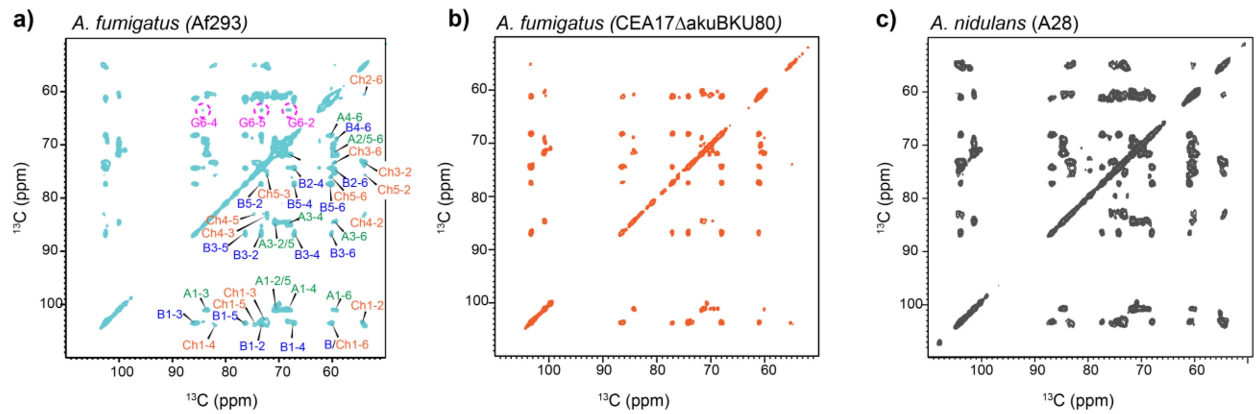


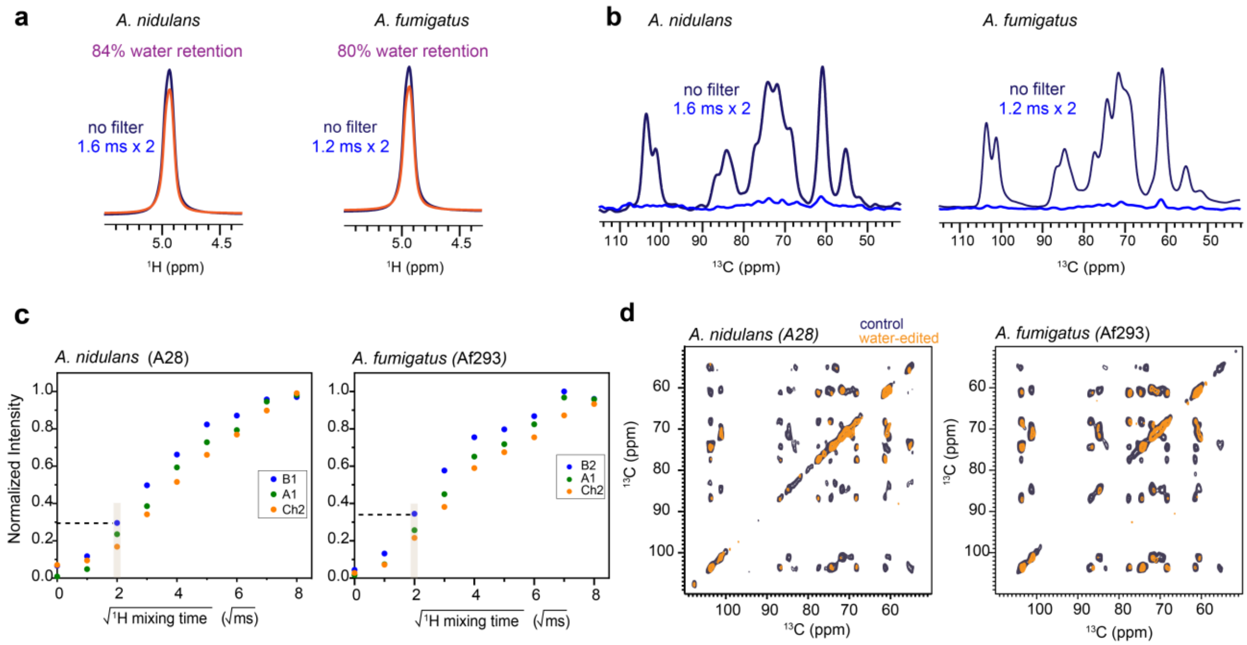
Supplementary Figure 1. Overlay for showing the zoom spectra of carbohydrate region. The spectra are color coded to show the black spectra for *A. nidulans* and orange for *A. fumigatus*. (**a**) Carbohydrates showing the highly mobile glucans obtained by INEPT experiment. (**b**) The semi mobile glucans probed by 2 s DP spectra and (**c**) The quantitative glucans represented by the 35 s DP spectra (**d**) The rigid molecules probed by CP experimentation.



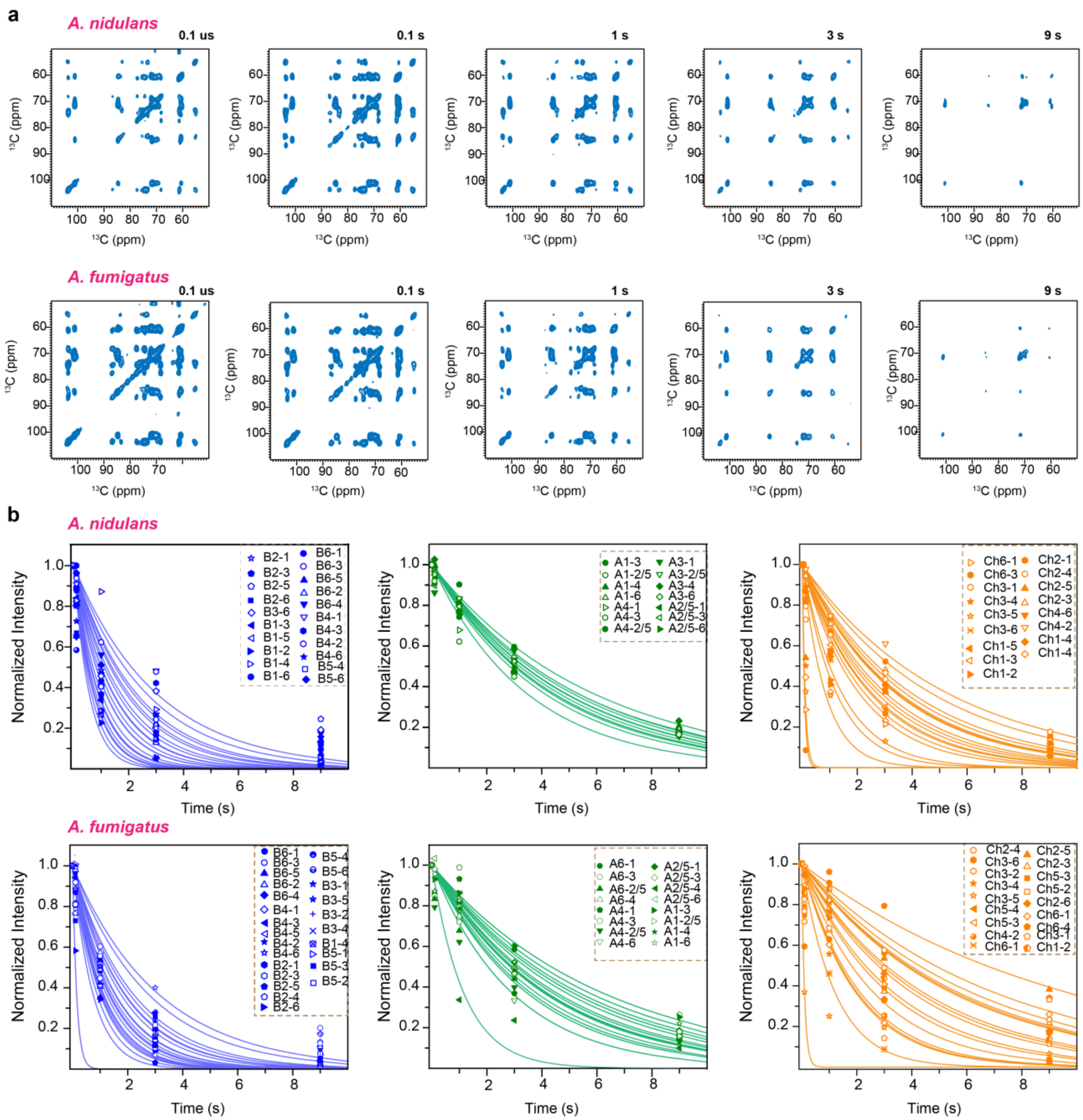
Supplementary Figure 2. Change is the mobile carbohydrates in different *Aspergillus* fungi. (**a**) The 2D ^{13}C-DP INADEQUATE spectra showing all the mobile glucans. (**b**) The mobile spectra representing Af293 strains of *A. fumigatus* showing no ^{13}C bond for GalNAc, (C1- C2) and GalN, (C1-C2). (**c**) The mobile spectra representing for CEA17ΔakuB^{KU80} with the signals for GalNAc(C1-C2) and GalN(C1-C2). The spectra of A28 and Af293 were measured at 15 kHz and CEA17ΔakuB^{KU80} was measured at 12 kHz.



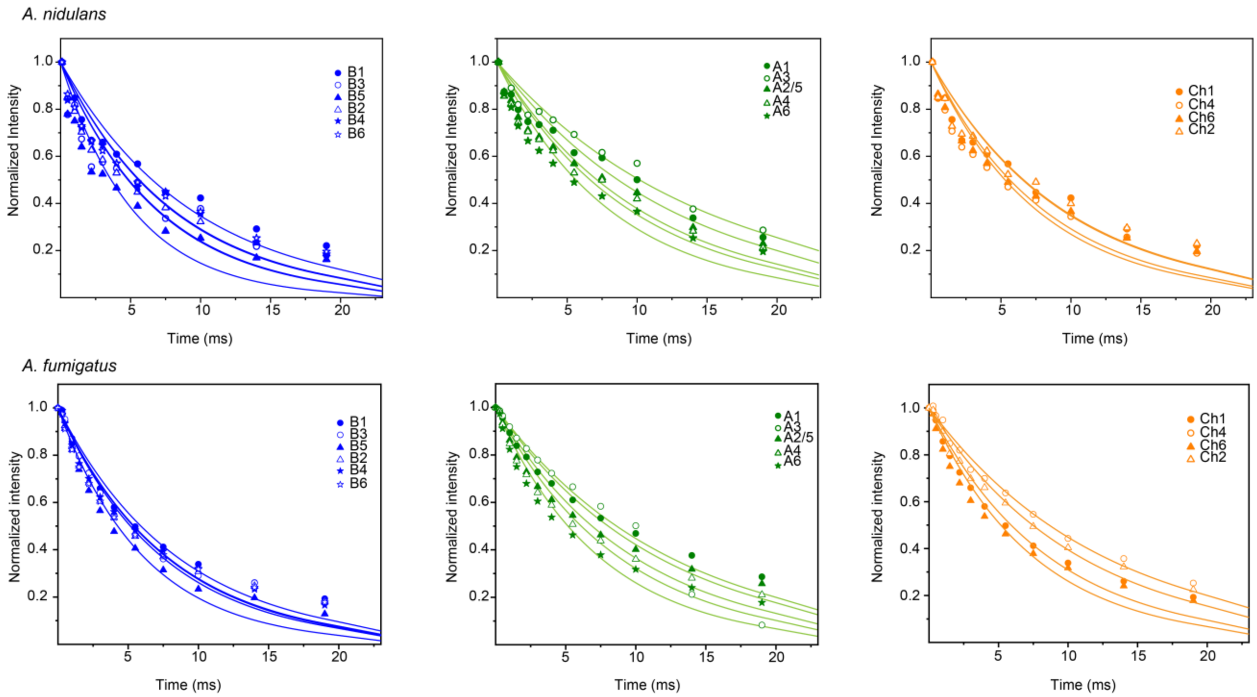
Supplementary Figure 3. β-1,3/1,4-glucose present in Af293. (**a**) 2D ^{13}C-^{13}C CORD spectra showing the rigid components in *A. fumigatus* (Af293) (turquoise) and (**b**) 2D ^{13}C-^{13}C CORD spectra showing the rigid components for *A. fumigatus* (CEA17ΔakuB^{KU80}) in orange spectra. (**c**) 2D ^{13}C-^{13}C CORD spectra showing the rigid components for *A. nidulans* (A28) in gray spectra. All measurements were obtained using 800 MHz NMR spectrometer.



Supplementary Figure 4. Water-edited analysis for *Aspergillus* cell wall. (**a**) Proton spectra illustrating the retention of 84% of water magnetization in *A. nidulans* (A28) and 80% in *A. fumigatus* (Af293) after the ^{1}H-T$_2$ filter. (**b**) Overlay of the ^{13}C CP spectra where T$_2$ is 1.6 ms × 2 and T$_2$ is 1.2 ms × 2 with no spin diffusion presented in *A. nidulans* (A28) and *A. fumigatus* (Af293) respectively. The ^{1}H-T$_2$ filtered resulted in a loss of 90% and 89% of the water magnetization in *A. nidulans* (A28) and *A. fumigatus* (Af293), respectively. (**c**) The water to build up curve illustrated for each glucan type which are color coded blue, green and orange for β-1,3-glucan, α-1,3-glucan and chitin respectively. (**d**) Water-edited intensities of carbohydrate carbon sites in *A. nidulans*, A28 (left) and *A. fumigatus*, Af293 (right). All the measurements were taken on a 400 MHz spectrometer at 10 kHz MAS and 280 K.



Supplementary Figure 5. 2D ^{13}C-^{13}C spectra for measuring ^{13}C-T_1 relaxation. a, The 2D ^{13}C-^{13}C T_1 relaxation **s**pectra obtained for examining cellular mobility in *A. nidulans* (top) and *A. fumigatus* (bottom). The spectra are organized in a sequence showing the varying z-filter duration durations; 0 s, 0.1 s, 1 s, 3 s, and 9 s. **b,**^{13}C-T_1 relaxation curves of *Aspergillus* cell wall polysaccharides**.** The 2D ^{13}C-T_1 relaxation curves are displayed for *A. nidulans* (top) and *A. fumigatus* (bottom). The data are acquired on a 400 MHz (9.4 Tesla) spectrometer with a 10 kHz MAS and at 298 K. The best fit is determined using a single exponential equation. β-1,3-glucan are represented by the blue curves while the α-1,3-glucan and chitin are represented as green and orange curves respectively. Symbols are used for assigning different carbons in the polysaccharides.



Supplementary Figure 6. ^{1}H-T$_{1\rho}$ relaxation curves of *Aspergillus* cell wall polysaccharides. The ^{1}H-T$_{1\rho}$ relaxation curves are displayed for *A. nidulans* (top) and *A. fumigatus* (bottom). The data are acquired on a 400 MHz (9.4 Tesla) spectrometer with a 10 kHz MAS. The best fit is determined using a single exponential equation. β-1,3-glucan are represented by the blue curves while the α-1,3-glucan and chitin are represented as green and orange curves respectively. Symbols are used for assigning different carbons in the polysaccharides.

Supplementary Table 1: Salt Solution concentration and minimal media components for *A. nidulans* (A28) and *A. fumigatus* (Af293) culture.

Minimal Media	
Salt components	g/L
$NaNO_3$	120.0
KCl	10.4
$MgSO_4.7H_2O$	10.4
KH_2PO_4	120
K_2HPO_4	20.9
Glucose	10.0
Trace element solution	1ml

Trace Elements	g/100ml
$CoCl_2.6H_2O$ g	0.16
$CuSO_4 \cdot 5H_2O$	0.16
MnCl2 ·4H2O	0.5
$(NH_4)6Mo_7O_{24}.4H_2O$	0.11
$Na_2EDTA \cdot 4H_2O$	0.11
ZnSO4 ·7H2O	2.2
H_3BO_3	1.1
$FeSO_4.7H_2O$	0.5

Supplementary Table 2. The average cell wall thickness of the three similar fungi. Results are described as the mean and the standard deviation of 10 individual cells with n=100 measurements in each fungal cell sample. Statistical analysis was performed using one-tailed, paired t-test at 95% confidence level ($p < 0.05$).

Sample	*A. nidulans* A28	*A. fumigatus* Af293	*A. fumigatus* CEA17Δ*akuB*^{KU80}
Average cell wall thickness (nm)	191 ±16	180 ± 23	206 ± 54

Supplementary Table 3. Solid- state NMR experimental parameters for *A. nidulans and A. fumigatus* The experimental parameters include the ^{1}H Larmor frequency, total experiment time (t), recycle delay (d1), number of scans (NS), The number of points for the direct (td2) and indirect (td1) dimensions, the acquisition time of the direct dimension (aq2) and the evolution time of indirect dimension (aq1), mixing time (t_m), and T filter times (t_z). * Indicates the water-polysaccharide spin diffusion and the DARR mixing time.

	Samples	Experiments	(Temp K)	B_0 (T)	ν_{MAS} (kHz)	d1 (s)	NS	td2	td1	aq2 (ms)	aq1 (ms)	t_m (ms)	t_z
1D	A28 Af293	CP	298	18.8	15	2	1024	3600		18			
	A28	DP	298	18.8	15	2 35	512	3600		18			
	Af293		293		13.5	2 30	128 32	4096		29			
	A28 Af293	Water-edited	280	9.4	10	2	512	2000		16		0, 1, 4,9,16,25,36,49,64, 81,100	
	A28 Af293	^{1}H $T_{1\rho}$ relaxation	298	9.4	10	2	512	1400		16			SL (0.1-19 ms)
	A28 Af293	INEPT	298	18.8	15	3.5	1024	3200		16			
			293		13.5	3.0	64	4400		30.8			
2D	A28 Af293	2D CORD	298	18.8	15	2	32	3200	400	16	5.2	50	
	A28 Af293	CP *J* INADEQUATE	298	18.8	15	2	32	2800	472	14	5.2		
	A28 A293	DP *J* INADEQUATE	298	18.8	15	2	32	2800	472	14	5.2		
	A28 Af293	Pseudo 3D ^{13}C-T_1	298	9.4	10	2	32	2000	100	16	5.5	50	(10^{-7}, 0.1, 1, 3, 9) s
	A28 Af293	Water-edited control	280	9.4	10	2	64	2000	1	16	5.5	0/50*	T_2 10^{-4} ms
	A28 Af293	Water-edited	280	9.4	10	2	64	2000	1	16	5.5	4/50*	T_2 1.6 ms T_2 1.2 ms
DNP	A28	DNP CP	92	14.1	10	2	8	2048	1	10.24			
		N(CA)CX		14.1	10	3.64	32	1700	50	12.69	3.13	100	
				14.1	10	3.64	32	1700	50	12.69	3.13	3000	
		PAR		14.1	10	3.64	16	1800	400	13.44	7	2	
				14.1	10	3.64	16	1800	400	13.44	7	20	

Supplementary Table 4. Experimental parameters used for proton detection experiments. All experiments were performed on 800 MHz (18.8 T) spectrometer and with the MAS frequency of 40 kHz.

Expt 2D	Samples	Temperature (K)	CP duration (μs)		D1	NS	Td2	Td1	Aq2 (ms)	aq2 (ms)	Decoupling power	Water suppression (ms)	*J*-evolution (ms)
			t_{cp1}	t_{cp2}									
hCH	A28 Af293 CEA17Δ*akuB*^{KU80}	304 291 293	200	50	3	8	1204	512	19.98	6.4	slpTPPM [1] (rf 10 kHz)	MISSISSIPI [2] (total duration) 200	----
refINEPT-HSQC	A28 Af293 CEA17Δ*akuB*^{KU80}	304 291 293	-	-	3	16	2408	768	39.9	9.6	WALTZ-16 [3]	200	2

Supplementary Table 5. ^{13}C and ^{15}N chemical shift (ppm) of biomolecules in the cell wall in *A. nidulans* (A28). Underline represents the ^{13}C connectivity with ambiguity and the (-) denotes unidentified.

Biomolecule		**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**N**	**Experimental Methods**	**Cell Wall Portion**	**References**
β-1,3-glucan		103.6	74.3	86.6	68.1	77.4	61.1		^{13}C-^{13}C CORD	Rigid	Shim et al. 2007[4]
α-1,3-glucan (A^a)		101.1	71.8	84.5	69.8	71.7	60.2				Bhanja et al 2014[5]
α-1,3-glucan (A^b)		100.0	71.6	-	-	71.6	-				
Chitin	a	104.0	55.2	73.5	83.1	75.9	60.7	126.2	CORD-^{13}C-^{13}C ^{15}N-^{13}C N(CA)CX		Fontaine et al. 2011[6]
	b	103.8	55.6	73.7	83.4	76.1	61.4	123.3			
	c	103.5	54.5	73.6	83.0	75.4	60.9	125.5			
	d	103.6	55.9	73.1	82.1	76.1	61.6				
$Mn^{1,2}$		101.1	78.2	71	67.4	73.5	61.4		^{13}C-^{13}C *J*- DP INADEQUATE	Mobile	Latge et al. 1994[7]
$Mn^{1,6}$		102.5	70.8	73.3	67.4	73.3	66.2				Chakraborty et al. 2021[8]
Gal*f*		107.4	81.7	77.1	82.7	71.8	63.6				
$Gal^a p$		92.6	72.1	70.2	73.3	72.1	61.2				
$Gal^b p$		96.7	75.0	76.6	70.4	72.7	61.6				Poulhazan et al. 2021[9]
GalN		91.5	54.6	71.6	81.8	-	-				Fontaine et al. 2011[6, 10]
GalNAc		95.6	57.3	75.2	76.5	-	-				

Supplementary Table 6. ^{13}C chemical shifts (ppm) of biomolecules in the cell wall of *A. fumigatus* (Af293). Underline represents the ^{13}C connectivity with ambiguity and the (-) denotes unidentified.

Biomolecule		C1	C2	C3	C4	C5	C6	Experimental Methods	Cell Wall Portion	References
β-1,3-glucan		103.6	74.3	86.6	68.1	77.3	61.1	^{13}C-^{13}C CORD	Rigid (Fontaine et al. 2011[6])	Shim et al. 2007[4]
β-1,4-glucan		103.2	69.4	72.2	85.3	74.3	63.3			Kang et al. 2018[11]
α-1,3-glucan (A[a])		101.1	71.8	84.5	69.8	71.7	60.2			Bhanja et al 2014[5]
α-1,3-glucan (A[b])		100.0	71.5	-	-	71.5	-			
Chitin	a	104.0	55.2	73.5	83.1	75.9	60.7	CORD-^{13}C-^{13}C		Fontaine et al. 2011[4]
	b	103.8	55.6	73.7	83.4	76.1	61.4			
Mn[1,2]		101.1	78.2	71	67.4	73.5	61.4	^{13}C-^{13}C *J*- DP INADEQUATE	Mobile	Latge et al. 1994[7]
Mn[1,6]		102.5	70.8	73.3	67.4	73.3	66.2			Latge et al. 1994[7] Chakraborty et al. 2021[8]
Gal*f*		107.4	81.7	77.1	82.7	71.8	63.6			
Gal[a]*p*		92.6	72.1	70.2	73.3	72.1	61.2			
Gal[b]*p*		96.7	75.0	76.6	70.4	72.7	61.6			Poulhazan et al. 2021[9]
GalN		91.5	54.4	71.6	81.8	-	-			Fontaine et al. 2011[6]
GalNAc		95.4	57.2	75.2	76.5	-	-			

Supplementary Table 7. Relative molar composition of *Aspergillus* cell wall. Molar composition of rigid and mobile components were calculated using the integrals of well-resolved cross peaks of β-1,3-glucan, α-1,3-glucan, and chitin in 2D ^{13}C-^{13}C CORD spectra and 2D ^{13}C-^{13}C *J*-INADEQUATE. The results were already normalized by number of scans. (-) is the notation for not detected.

	Polysaccharide		A28			A293			CEA17Δ*akuB*^{KU80}		
			Cross-peaks	%	Error %	Cross-peaks	%	Error %	Cross-peaks	%	Error %
Rigid Composition	β -1,3-glucan		B1-3, B1-5, B1-2, B1-4, B2-4, B2-6, B3-5, B3-2, B3-4, B3-6, B5-2, B5-4, B5-6	25.2	0.1	B1-3, B1-4, B1-2, B1-6, B1-5, B3-5, B3-2, B3-6, B5-6, B5-4, B2-4, B4-6	31.6	7.2	B1-3, B1-4, B1-5, B3-4, B3-5, B3-2, B5-4, B5-2, B2-4	50%	6%
	β -1,4-glucose		G1-4, G1-2, G1-6	/	/	G1-6, B3-6, G4-5	5.2	1.6	/		
	α-1,3-glucan		A1-2/5, A1-3, A1-4, A3-4, A3-2/5, A2/5-6, A2/5-4	44	0.9	A1-4. A1-2/5, A1-4, A1-6, A3-2/5, A2/5-6	40.4	10.5	A1-4. A1-2/5, A3-2/5, A3-4	42%	7%
	Chitin		Ch1-4, Ch1-5, Ch1-2, Ch3-2, Ch4-2, Ch4-3, Ch4-5, Ch5-2, Ch5-3, Ch3-2	31.2	0.1	Ch1-4. Ch1-2, Ch4-6, Ch4-5, Ch4-3, Ch2-4, Ch2-6	22.4	4.8	Ch1-4, Ch1-2, Ch1-5, Ch1-3, Ch4-2, Ch4-2, Ch4-3, Ch4-5, Ch5-3, C3-2, Ch5-2	8%	3%
composition	GM	Gal*f*	Gal*f* 1-2, Gal*f* 2-3, Gal*f 5-6*	13	3	Gal*f* 1-2, Gal*f* 2-3, Gal*f* 4-5, Galf 3-4, Gal*f 5-6*	24.8	6.7	Gal*f* 1-2, Gal*f* 3-4	20%	2%
		$Mn^{1,2}$	$Mn^{1,2}$1-2, $Mn^{1,2}$2-3, $Mn^{1,2}$ 3-4, $Mn^{1,2}$ 5-6,	7	2	$Mn^{1,2}$1-2, $Mn^{1,2}$ 3-4, $Mn^{1,2}$ 5-6	10.9	2.5	$Mn^{1,2}$1-2, $Mn^{1,2}$4-5	24%	1%
		$Mn^{1,6}$	$Mn^{1,6}$ 3-4, $Mn^{1,6}$5-6,	8	2	$Mn^{1,6}$1-2, $Mn^{1,6}$ 3-4, $Mn^{1,6}$2-3	20.4	6.8	$Mn^{1,6}$ 1-2, $Mn^{1,6}$ 3-4,	5.1%	0.3%
	GAG	Gal*p*	Gal1-2, Gal3-4, Gal5-6, Gal2-3	29.7	9	Gal1-2, Gal3-4, Gal5-6	20.2	6.0	Gal1-2, Gal3-4	27%	2%
		GalNAc	GalNAc 1-2, GalNAc 3-4, GalNAc 2-3	20	10	GalNAc 1-2, GalNAc 3-4	11.3	5.2	GalNAc 1-2, GalNAc 3-4	13%	3%
		GalN	GalN1-2, GalN3-2	16.3	5	GalN	1.9	4.1	GalN 1-2, GalN3-4	6%	1%
other	B1-2			4	1	B-2, B3-6	9.0	0.4	A1-2, A4-5	0.83%	0.1%
	A2/5-6			2	1	A2/5-4	1.5	0.3	B1-2, B4-5	4%	1%

Supplementary Table 8. Intermolecular interaction of polysaccharides in *A. nidulans* cell wall. The chemical shifts for the two dimensions of the spectra (ω_1 and ω_2), The cross-peak assignments and the spectral type are documented in the table below.

Cross Peak	Chemical Shift (ω_1)	Chemical Shift (ω_2)	2 ms PAR	20 ms PAR	NCACX (3 s PDSD)
ChMe-Me'	23.3	22.2		x	
ChMe'-Me	21.5	22.2		x	
ChMe-Ch4	82.3	22.4		x	
Ch1-ChO	103.3	174.0		x	
A2-Ch4	83.0	71.1		x	
A5-Ch4	83.0	71.1		x	
A4-Ch2	60.0	83.4		x	
A1-Ch1	103.6	101.1		x	
A1-Ch2	55.0	101.1		x	
A1-Ch4	82.3	101.4	x	x	
A1-Ch6	60.1	100.1		x	
A2-Ch4	71.2	83.0		x	
A5-Ch4	71.2	83.0		x	
A6-ChMe	60.2	22.3		x	
A2-ChCO	72.1	176.8		x	
A5-ChCO	72.1	176.8		x	
A3-ChMe	84.0	22.6		x	
A1-ChMe	101.4	22.3		x	
Ch2-A1	101.3	55.8		x	
Ch2-A6	60.0	54.5		x	
Ch3-A1	73.3	100.1		x	
Ch4-A2	83.3	70.2	x	x	
ChMe-A1	101.4	23.4		x	
ChMe-A3	84.0	22.4		x	
CHMe-A2	82.3	22.6		x	
CHMe-A5	71.6	22.6		x	
ChN-A3	84.8	126.5			x
A6-B6	62..2	60.0	x	x	
A1-B2	74.4	101.3		x	
A1-B4	68.3	101.1	x	x	
A1-B6	68.8	100		x	
B5-A6	60.07	77.2		x	
B6-A1	100.1	61.2		x	
B6-A6	62.2	60.0		x	
B1-ChMe	103.6	22.4			
B6-ChMe	61.7	22.3		x	
B5-Ch6	60.7	77.2		x	
Ch2-B6a	61.8	55.0		x	
Ch4-B4	68.6	83.2		x	
ChN-B3	86.1	126.1			x
ChN-B5	77.5	126.4			x
B5-ChMe	77.2	22.5		x	
ChMe-B5	22.4	77.7		x	
ChMe-B6	61.6	21.5		x	
B2-ChCO	74.8	177.1		x	
B1-ChCO	103.3	177.2		x	

Supplementary Table 9: Water-edited intensities of the polysaccharides. The intensity ratios are obtained by comparing the peak intensities between water-edited and the control 2D spectra of the *Aspergillus* cell walls. The error bars represent propagated standard deviations from NMR signal-to-noise ratios, with an error margin typically below 10%,

β-1,3-glucan		A28	Af293
103 line	**103.6**	0.25±0.03	0.31±0.02
	86.7	0.4±0.1	0.40±0.09
	77.4	0.3±0.1	0.5±0.1
	74.3	0.22±0.05	0.32±0.03
	68.1	0.3±0.1	0.37±0.06
	61.1	0.19±0.09	0.32±0.08
86 line	**103.5**	0.3±0.1	0.23±0.06
	86.6	0.4±0.1	0.21±0.08
	77.3	0.40±0.07	0.3±0.1
	74.3	0.24±0.09	0.25±0.07
	68.2	0.4±0.1	0.24±0.07
	61.1	0.3±0.1	0.31±0.09
77 line	**103.5**	0.4±0.1	0.22±0.07
	86.71	0.4±0.1	0.16±0.09
	77.1	0.29±0.04	0.34±0.04
	74.4	0.24±0.09	0.22±0.06
	68.1	0.28±0.09	0.25±0.05
	61.2	0.33±0.06	0.24±0.05
74 line	**103.5**	0.24±0.04	0.25±0.03
	86.6	0.3±0.1	0.35±0.09
	77.2	0.32±0.08	0.07±0.02
	74.2	0.25±0.02	0.27±0.02
	68.2	0.32±0.08	0.24±0.05
	61.0	0.18±0.05	0.19±0.05
68 line	**103.6**	0.3±0.1	0.24±0.05
	86.6	0.30±0.07	0.26±0.08
	77.4	0.3±0.1	0.32±0.07
	74.3	0.4±0.1	0.22±0.05
	68.5	0.25±0.06	0.20±0.03
	61.1	0.29±0.09	0.21±0.05
61 line	**103.6**	0.25±0.09	0.24±0.05
	86.6	0.30±0.09	0.4±0.1
	77.5	0.32±0.09	0.34±0.05
	74.7	0.22±0.07	0.27±0.05
	68.3	0.21±0.07	0.21±0.03
	61.0	0.26±0.02	0.23±0.01
Average		**0.29**	**0.27**
n		**36**	**36**

α-1,3-glucan		A28	Af293
101 line	**101.1**	0.22±0.04	0.28±0.03
	84.5	0.3±0.1	0.20±0.07
	71.6	0.19±0.06	0.20±0.04
	69.6	0.3±0.1	0.22±0.06
	60.7	0.30±0.07	0.28±0.09
84 line	**101.1**	0.14±0.08	0.18±0.05
	84.5	0.22±0.05	0.25±0.05
	71.6	0.20±0.06	0.24±0.04
	70.2	0.24±0.08	0.18±0.06
	60.6	0.3±0.1	0.14±0.06
70 line	**100.9**	0.31±0.07	0.27±0.05
	84.5	0.20±0.09	0.30±0.08
	70.6	0.30±0.03	0.27±0.02
	60.8	0.30±0.06	0.27±0.05
71 line	**101.0**	0.21±0.06	0.18±0.03
	84.6	0.24±0.09	0.23±0.05
	71.4	0.27±0.03	0.22±0.02
	60.7	0.26±0.07	0.19±0.03
61 line	**101.1**	0.19±0.06	0.23±0.07
	84	-	0.24±0.08
	71.7	0.29±0.06	0.21±0.03
	61.0	0.26±0.02	0.23±0.01
		0.24	**0.23**
		21	**22**

Chitin		A28	Af293
103 line	**103.4**	0.26±0.03	0.32±0.03
	83.1	-	0.19±0.07
	61.1	0.20±0.09	0.32±0.08
	55.3		0.05±0.02
83 line	**103.6**	0.23±0.03	0.28±0.08
	83.7	-	0.3±0.1
	73.4	-	-
	60.3	-	0.26±0.08
	54.7	-	0.25±0.02
75 line	**103. 6**	0.23±0.08	0.23±0.09
	83.2	0.20±0.08	0.19±0.06
	75.1	0.21±0.05	0.18±0.03
	60.8	0.15±0.09	0.3±0.1
	54.8	0.17±0.08	0.15±0.03
73 line	**103.6**	0.27±0.08	0.25±0.03
	83.2	-	0.20±0.07
	60.9	0.20±0.08	0.18±0.06
	55.2	0.43±0.07	0.3±0.1
61 line	**103.6**	0.25±0.09	0.16±0.04
	61.0	0.26±0.01	0.23±0.01
	55.6	0.18±0.08	-
55 line	**103.8**	0.13±0.01	0.09±0.03
	83.2	-	0.12±0.03
	75.6	-	0.4±0.1
	73.2	0.14±0.06	0.3±0.1
	60.4	0.11±0.05	0.20±0.06
		0.20	**0.22**
		17	**26**

Supplementary Table 10. ^{1}H-T$_{1\rho}$ relaxation times of polysaccharides. A single exponential equation was used to fit the T$_1$ data $I(t) = e^{-t/T_I}$. A single exponential equation was used to fit the T$_{1\rho}$ data: $I(t) = e^{-t/T_{I\rho}}$. Error bars are standard deviations of the fit parameters.

Sample Type	Cross peaks	^{1}H-T$_{1\rho}$ (ms)	Average
A28	B1	9.4 ± 0.8	7.5 ± 0.7
	B3	7.0 ± 0.8	
	B5	5.2 ± 0.6	
	B2	7.0 ± 0.7	
	B4	8.0 ± 0.7	
	B6	8.1 ± 0.7	
	A1	12.5 ± 0.9	11.0 ± 0.8
	A3	14.7±0.9	
	A2/5	10.3 ± 0.8	
	A4	9.6 ± 0.9	
	A6	8.1 ± 0.7	
	Ch1	9.4 ± 0.8	8.7 ± 0.8
	Ch3	7.6 ± 0.7	
	Ch5	-	
	Ch6	8.1 ± 0.7	
	Ch2	9.6 ± 0.8	
Af293	B1	8.5 ± 0.4	7.5 ± 0.4
	B3	7.8 ± 0.4	
	B5	6.1 ± 0.3	
	B2	7.4 ± 0.4	
	B4	7.8 ± 0.4	
	B6	7.4 ± 0.5	
	A1	12.5 ± 0.6	10.1 ± 0.6
	A3	11.6 ± 0.7	
	A2/5	10.0 ± 0.7	
	A4	8.8 ± 0.6	
	A6	7.4 ± 0.5	
	Ch1	8.5 ± 0.4	9.8 ± 0.5
	Ch4	12.5 ± 0.4	
	Ch6	7.4 ± 0.5	
	Ch2	10.8 ± 0.5	

Supplementary Table 11. 2D ^{13}C-T_1 relaxation times of polysaccharides within *Aspergillus* cell walls. Data are presented for the *A. nidulans* (A28) and *A. fumigatus* (Af293). The bold values indicate average measurements for each polysaccharide within each sample. The relaxation times were obtained through 2D ^{13}C-^{13}C correlation experiments and are fit using single exponential equation $I(t) = e^{-t/T_I}$. Error bars are standard deviations of the fit parameters.

β-1,3-glucans cross peaks	*A. nidulans* A28	*A. fumigatus* Af293	Chitin Cross peaks	A28	Af293	α-1,3-glucans cross peaks	A28	Af293
B6-1	1.4±0.3	1.4±0.5	Ch2-1	-	5.4±0.5	A1-3	5.8±0.4	6.3±0.2
B6-3	1.9±0.3	1.7±0.2	Ch2-4	2.9±0.9	3.1±0.9	A1-2/5	5.1±0.2	4.6±0.3
B6-5	1.2±0.2	1.0±0.2	Ch2-3	-	3.3±0.7	A1-4	4.5±0.4	4.2±0.2
B6-2	1.2±0.4	1.1±0.2	Ch2-3	3.9±0.4	3.3±0.7	A1-6	4.4±0.4	5.2±0.7
B6-4	1.9±0.7	1.5±0.4	Ch4-6	2.9±0.5	-	A3-1	5.3±0.7	-
B4-3	2.0±0.7	2.1±0.2	Ch4-2	4.7±0.6	-	A3-2/5	4.9±0.2	-
B4-2		1.9±0.7	Ch1-4	2.7±0.6	-	A3-4	5.1±0.5	-
B4-6	1.6±0.7	2.8±0.7	Ch1-5	0.8±0.5	2.6±0.4	A3-6	4.4±0.3	-
B2-1	1.7±0.1	1.7±023	Ch1-3	2.5±0.5	-	A2/5-1	4.8±0.1	4.9±0.2
B2-3	1.5±0.2	1.3±0.2	Ch1-2	2.3±0.3	4.2±0.8	A2/5-3	4.6±0.4	5.4±0.3
B2-5	1.0±0.5	0.9±0.2	Ch6-1	1.4±0.3	2.1±0.7	A2/5-6	4.4±0.4	4.4±0.3
B2-6	0.9±0.3	0.7±0.3	Ch3-1	2.1±0.5	2.0±0.4	A4-1	4.3±0.6	-
B5-4	0.8±0.1	1.5±0.3	Ch5-3	-	4.4±0.4	A4-3	4.1±0.4	5.8±1.4
B5-1	-	1.3±0.2	Ch5-2	-	3.7±0.4	A4-2/5	3.4±0.6	2.9±0.7
B5-3	-	1.7±0.5	Ch3-4	3.7±0.9	2.6±0.6	A6-3		3.8±0.6
B5-6	1.3±0.2	1.5±0.5	Ch3-5	1.2±0.7	-	A6-2/5		3.5±0.6
B3-6	2.2±0.7	-	Ch3-6	0.1±0.5	0.1±0.04	A6-1		5.2±0.7
B1-3	0.9±0.2	-	Ch3-2	2.6±0.6	2.0±0.8	A6-4		4.2±0.4
B1-5	0.8±0.2	-	Ch4-1		4.1±0.6	A4-6		3.6±0.7
B1-4	3±0.7	1.3±0.1	Ch4-3		4.0±0.4	A2/5-4		1.2±0.3
B1-6	0.6±0.3	-	Ch1-6		1.2±0.2			
B1-6	0.6±0.3	-						
B3-1	-	1.2±0.1						
B3-5	-	1.6±0.3						
Average	**1.39**	**1.48**		**2.41**	**3.0**		**4.65**	**4.35**
n	19	19		14	16		14	15

Supplementary Table 12. ^{1}H and ^{13}C chemical shifts of *A. nidulans* polysaccharides. For each carbon site, the ^{13}C and ^{1}H chemical shifts are shown in the top and bottom rows, respectively. All ^{1}H proton chemical shifts were following Safeer et al. 2023[12]. Chemical shifts of NRa, NRb, Rb, B, and A are following Ehren et al. 2020[13]. Chemical shifts of Mn, Galf, Galp, GalN, GalNAc are following values from Latgé et al. 1994[7]and Chakraborthy et al. 2021[8].

Biomolecule	C1	C2	C3	C4	C5	C6	C8
α-1,3-glucan	101.1 5.4	71.8 3.7	84.5 3.6	69.8 3.7	71.7 3.8	60.9 3.8	---
β-1,3-glucan	103.6 4.9	74.3 3.6	86.7 3.6	68.1 3.4	77.3 3.4	60.9 3.8	---
Chitin	103.7 4.0	55 3.8	73.5 3.6	83 3.6	75.6 3.6	60.9 3.8	22.8 2.1
NR^{a}	98.6 5.0						---
R^{b}	98.4 4.9	---	---	---	---	---	---
NR^{b}	103.1 4.7	73.8 3.9					
B	103.1 4.7	73.8 3.9					
Mn^{1,2}	101.1 5.2	78.6 4.0	71.1 3.8	67.4 3.7	73.7 4.1	61.7 3.8	---
Mn^{1,6}	102.6 5.0	70.7 3.9	73.2 4.1	67.5 3.7	73.7 4.1	66.3 3.7	---
Gal*f*	107.4 5.2	81.7 4.1	77.7 4.0	83.2 4.0	71.5 3.8	63.5 3.7	---
Gal^{a}*p*	92.6 5.2	72.1 3.5	70.2 3.4	---	72.1 3.8	61.3 3.8	---
Gal^{b}*p*	96.7 5.4	-	76.5 3.4	70.3 3.4	-	61.6 3.8	---
GalN		54.4 3.2		81.8 4.1	-	-	
GalNAc	96.4/95.6 4.6	57.2 3.7	---	76.9 4.0	-	-	22.7 2.0

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