

# Supplementary Information

## Solid-State NMR Analysis of Unlabeled Fungal Cell Walls from *Aspergillus* and *Candida* Species

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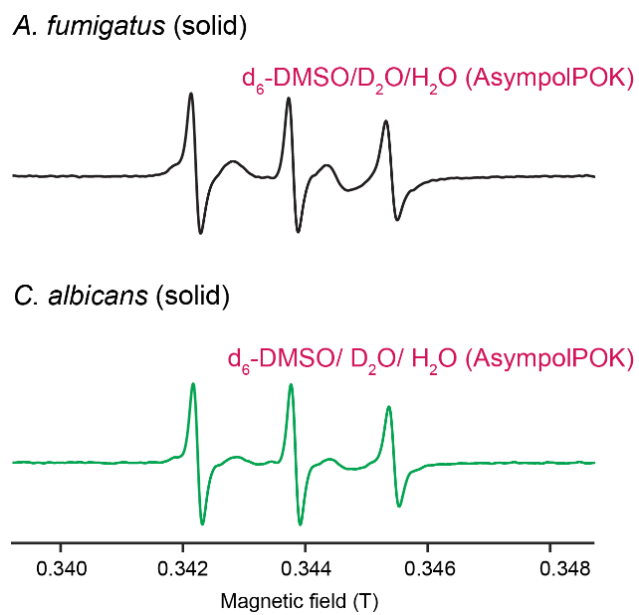
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**Supplementary Figure 1.** Room-temperature EPR spectra of AsympolPOK at 9.6 GHz.

**Table S1. Experimental parameters used for MAS-DNP and ssNMR on intact fungal cells.** Abbreviations are used for  $^1\text{H}$  Larmor frequency ( $\omega_{0, 1\text{H}}$ ), total experimental time (t), recycle delay (d1), number of scans (NS), number of points in F2 (td2), number of increments in F1 (td1), acquisition time during t2 for F2 (aq2), and indirect acquisition time for F1 (aq1). Not applicable (-). Experiments were collected on either a 600 MHz/395 GHz MAS-DNP, an 800 MHz ssNMR, or a 400 MHz ssNMR.

Sample	Experiment	$\omega_{0, 1\text{H}}$ (MHz)	t (hr)	MAS (kHz)	NS	d1 (s)	td2	td1	aq2 (ms)	aq1 (ms)
<i>C. albicans</i> (solid)	1D $^1\text{H}$ (MW on/off)	600	2.2	8	2	1.0	-	4096	-	5.7
	1D $^{13}\text{C}$ (MW on/off)		63.7	64	3.5	-	1024	-	5.7	
	1D CP		254	10.5	256	3.5	-	1024	-	5.7
	CP refocused dipolar (SPC5) INADEQUATE		20	256	3.5	2048	80	17.2	1.2	
<i>C. albicans</i> (liquid)	1D $^1\text{H}$ (MW on/off)	600	2.2	8	2	1.0	-	4096	-	5.7
	1D $^{13}\text{C}$ (MW on/off)		72.8	64	4.0	-	1024	-	5.7	
	CP refocused dipolar (SPC5) INADEQUATE		5.6	10.5	256	4.0	2048	80	17.2	1.2
	DP refocused J- INADEQUATE	800	0.4	13.5	8	1.5	2600	120	19.4	1.2
	CP refocused dipolar (SPC5) INADEQUATE	400	5.2	7.5	112	1.7	1800	100	18.0	6.2
<i>A. fumigatus</i> (solid)	1D $^1\text{H}$ (MW on/off)	600	2.2	8	2	1.0	-	4096	-	5.7
	1D $^{13}\text{C}$ (MW on/off)		27.3	10.5	64	1.5	-	1024	-	5.7
	$^{13}\text{C}-T_1$	400	120	14	10-40k	2.0	-	1800	-	18.0
	CP refocused dipolar (SPC5) INADEQUATE	600	18.4	10.5	1024	1.3	2048	50	17.2	0.8
	DQF-DARR	600	30	10.5	32/64	5.0	2048	200-300	17.2	0.3
<i>A. fumigatus</i> (liquid)	CP J-INADEQUATE	800	9	13.5	16	2.0	2400	1024	17.9	10.2
	$^{13}\text{C}-T_1$	400	120	14	24-40k	2.0	-	1800	-	18.0

**Table S2. Parameters used for the fit of  $^{13}\text{C}$  MultiCP spectra in Figure 6A.** unk: unknown or unresolved peaks. The error was estimated using the Monte Carlo error estimation of Dmfit.

$\Delta$ (ppm)	assignment	amplitude	width (ppm)	integral (%)
<i>C. albicans</i> liquid culture				
103.1	B1	7511	1.22±0.03	5.7±0.2
85.9	B3	8000	1.05±0.03	2.26±0.07
78.1	B5	8196	1.4±0.1	1.2±0.2
74.4	B2	8305	0.83±0.01	20.1±0.3
68.6	B4	8466	0.56±0.02	18±2
103.4	Ch1	7482	1.43±0.03	3.9±0.2
82.9	Ch3	8115	0.88±0.07	2.1±0.2
54.3	Ch2	8861	1.54±0.07	0.54±0.06
101.8	Mn <sup>1,2</sup> 1 <sup>a</sup>	7564	0.83±0.02	4.9±0.2
79.6	Mn <sup>1,2</sup> 2 <sup>a</sup>	8161	1.11±0.09	1.5±0.2
70.5	Mn <sup>1,2</sup> 3 <sup>a</sup>	84116	0.66±0.03	13±2
98.0	Mn <sup>1,2</sup> 1 <sup>b</sup>	7651	1.05±0.07	1.33±0.09
72.1	Mn <sup>1,6</sup>	8365	0.73±0.03	9±2
62.3	unk	8642	0.82±0.02	10.9±0.2
59.26	unk	8725	0.80±0.02	5.0±0.2
<i>C. albicans</i> solid culture				
103.1	B1	7478	0.9±0.4	4.3±0.5
85.9	B3	8002	0.63±0.03	2.3±0.1
78.1	B5	8188	0.64±0.06	2.1±0.5
74.4	B2	8305	0.53±0.01	21.1±0.5
68.6	B4	8509	0.62±0.09	4±1
103.4	Ch1	7514	0.95±0.03	4.3±0.4
82.9	Ch3	8130	0.55±0.04	3.4±0.5
54.3	Ch2	8775	0.11±0.01	2.5±0.1
101.8	Mn <sup>1,2</sup> 1 <sup>a</sup>	7570	0.74±0.03	3.4±0.2
69	Mn <sup>1,2</sup> 2 <sup>a</sup>	8453	0.51±0.03	12±1
70.5	Mn <sup>1,2</sup> 3 <sup>a</sup>	8397	0.44±0.02	17±2
98.0	Mn <sup>1,2</sup> 1 <sup>b</sup>	7646	0.89±0.08	1.1±0.2
62.3	unk	8641	0.56±0.08	11±4
59.26	unk	8715	0.8±0.1	1.8±0.5

**Table S3. The average cell wall thickness of *A. fumigatus* and *C. albicans*.** Results describe the average of 10 measurements from 15 biological replicates of each solid and liquid cultures.

Sample	Cell wall thickness (nm)	
	<i>A. fumigatus</i>	<i>C. albicans</i>
Solid culture	134±21	195±50
Liquid culture	159±35	182±68

**Table S4. DNP buildup time and enhancement factor.** The enhancement factor ( $\epsilon_{\text{on/off}}$ ) is obtained by comparing the intensity of spectra measured with microwave on and off. The concentration of biradicals, the culture condition (solid or liquid media), and the composition of the DNP juice (matrix) are also listed.

Sample	Radical	Matrix	DNP build-up time (s)	$\epsilon_{\text{on/off}}$
<i>A. fumigatus</i> (solid)	20 mM c-AsymPol-POK	d <sub>6</sub> -DMSO-D <sub>2</sub> O-H <sub>2</sub> O (1:8:1)	1.3	26
<i>A. fumigatus</i> (solid)	10 mM AMUPol,	d <sub>8</sub> -glycerol/D <sub>2</sub> O/H <sub>2</sub> O (6:3:1)	5.0	90
<i>C. albicans</i> (solid)	20 mM c-AsymPol-POK	d <sub>6</sub> -DMSO-D <sub>2</sub> O-H <sub>2</sub> O (1:8:1)	2.6	30
<i>C. albicans</i> (liquid)			3.1	26

**Table S5. <sup>13</sup>C chemical shifts of polysaccharides and proteins in fungal cell walls.** The units are in ppm. (/) unidentified. (-) not applicable.

Carbohydrate	Chemical shifts (ppm)								References
	C1	C2	C3	C4	C5	C6	CO	CH <sub>3</sub>	
β-1,3-glucan	103.6	74.2	87.5	68.3	77.2	61.5	-	-	Chakraborty et al. 2021 <sup>1</sup>
α-1,3-glucan	101	71.9	84.5	69.5	71.7	60.5	-	-	Chakraborty et al. 2021 <sup>1</sup>
β-1,6-glucan	103.9	74.6	76.6	70.6	75.7	69.6	-	-	Lowman et al. 2011 <sup>2</sup>
Chitin	103.3	55.5	72.9	83.0	75.7	60.9	174.8	22.6	Chakraborty et al. <sup>1</sup>
α-1,2-Mannose <sup>a</sup>	101.4	79.2	71.0	67.8	74.1	61.9	-	-	
α-1,2-Mannose <sup>b</sup>	99.1	79.5	71.4	67.8	74.1	61.9	-	-	
α-1,6-Mannose	102.9	71.1	/	/	72.0	66.6	-	-	
<b>Amino Acids</b>	<b>Cα</b>	<b>Cβ</b>	<b>Cγ</b>				<b>CO</b>		
Alanine	52.4	16.3					177.0		Fritzsching et al. 2013 <sup>3</sup>
Aspartic acid	54.8	38.3	/				175.9		
Glutamic acid	54.6	28.1	34.1				174.0		
Phenyl alanine	55.5	37.7	/				172.8		
Iso leucine	58.7	36.6	25.1	/	/		173.5		
Lysine	54.2	30.8	22.8		/	/	175.0		
Leucine	52.7	40.3	24.8		/	/	174.4		
Methionine	53.2	31.1	29.9			/	173.4		
Asparagine	51.1	36.6	/				172.6		
Proline	61.1	30.0	25.2				174.2		
Glutamine	53.7	27.4	31.7				173.4		
Arginine	53.9	28.7	25.0				173.7		
Threonine	59.5	67.4	/				172.4		
Valine	59.7	30.7	19.2	/			173.4		
Tryptophan	55.0	28.0	/	/	/	/	173.0		
Tyrosine	58.6	36.0	/	/	/	/	173.0		

**Table S6.  $^{13}\text{C}$ - $T_1$  relaxation times of polysaccharides.** The data are fit using bi-exponential equations:  $I(t) = e^{\frac{-t}{T_{1b}}}$  and  $I(t) = Ae^{\frac{-t}{T_{1a}}} + (1 - A)e^{\frac{-t}{T_{1b}}}$ , where A is a perfector. Error bars are standard deviations of fit parameters.

<i>A. fumigatus</i> (liquid culture)					
Assignment	$^{13}\text{C}$ (ppm)	A	$T_{1a}$ (s)	B	$T_{1b}$ (s)
B3	86	70 %	$0.6 \pm 0.1$	30 %	$5 \pm 3$
B5	77	40 %	$0.4 \pm 0.1$	60 %	$4 \pm 2$
B2	74	20 %	$0.01 \pm 0.01$	80%	$5 \pm 1$
Ch4	83	28 %	$0.20 \pm 0.01$	72%	$14 \pm 4$
Ch5	75	50 %	$0.0045 \pm 0.0001$	50 %	$7 \pm 2$
Ch2	55	43%	$0.17 \pm 0.06$	57 %	$13 \pm 2$
A1	101	20 %	$0.5 \pm 0.2$	80 %	$18 \pm 6$
A3	84	23 %	$0.8 \pm 0.2$	77 %	$23 \pm 10$
A2/5	71	23 %	$0.0010 \pm 0.0005$	77 %	$8 \pm 2$
<i>A. fumigatus</i> (solid culture)					
B3	86	10 %	$0.20 \pm 0.02$	90 %	$5 \pm 1$
B5	77	50 %	$0.8 \pm 0.3$	50%	$6 \pm 3$
B2	72	20 %	$0.300 \pm 0.001$	80%	$5.420 \pm 0.006$
B4	68	20 %	$0.0003 \pm 0.0001$	80%	$3.8 \pm 0.4$
Ch4	83	11%	$0.5 \pm 0.4$	89%	$20 \pm 6$
Ch5	75	11%	$0.11 \pm 0.05$	89%	$2.8 \pm 0.3$
Ch2	55	14 %	$0.14 \pm 0.01$	86 %	$10.8 \pm 0.3$
A1	101	30 %	$0.011 \pm 0.002$	60 %	$14 \pm 5$
A3	84	44 %	$0.6 \pm 0.1$	56 %	$14 \pm 4$
A2/5	71	60%	$0.348 \pm 0.009$	40 %	$9.7 \pm 0.6$



## Supplementary References

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