Solid-state NMR spectroscopy identifies three classes of lipids in *C. neoformans* melanized cell walls and whole fungal cells

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SUPPORTING INFORMATION

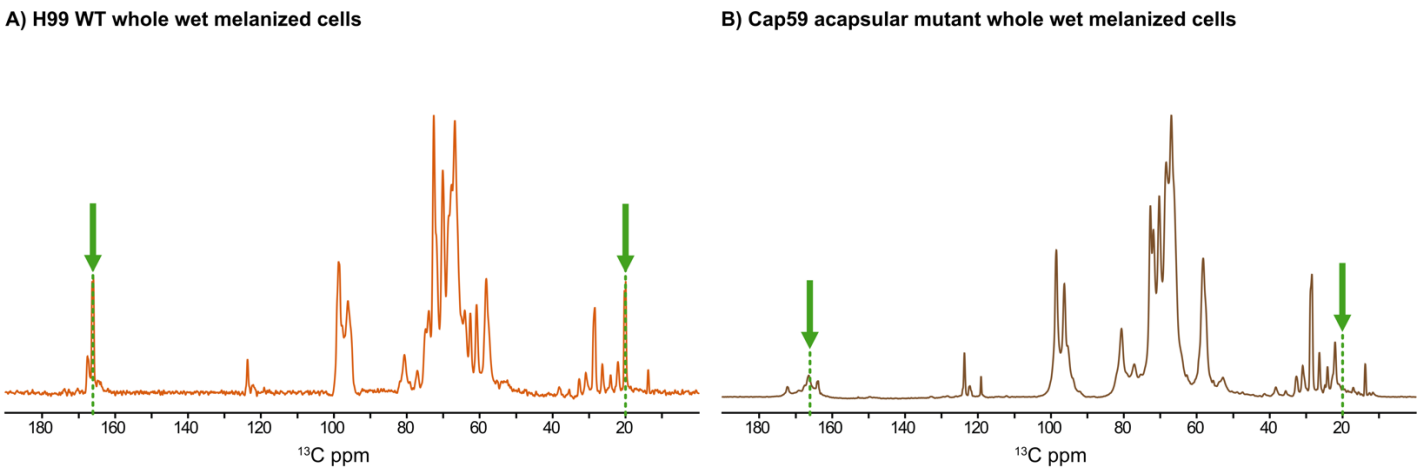
Supplementary Table 1. ^{13}C and ^{1}H chemical shifts corresponding to the lipids observed in the ^{13}C-^{13}C DP-INADEQUATE and ^{1}H-^{13}C INEPT-HETCOR spectra of [U-^{13}C]glucose-enriched melanin ghost and whole *C. neoformans* cell samples.

Constituent	Functional group	Assignment	^{13}C ppm	^{1}H ppm	References
Triglycerides	-COO	all FAs, C1	172.4	--	[21-30]
	-CH_2-COO	all FAs, C2	34.3	1.44	
	-CH_2-CH2-COO	all FAs, C3	25.0	1.57	
	-CH_2-CH_2-	all FAs, bulk methylene	29.3-29.9	1.26-1.29	
	-CH_2-CH=CH-	all unsaturated FAs, allylic	27.3	1.96	
	-CH=CH-	all unsaturated FAs, olefinic	128.1-130.0	5.26-5.29	
	-CH=CH-CH_2-CH=CH-	polyunsaturated FAs, bis-allylic	25.6	2.74	
	-CH_2-CH_2-CH_3	all FAs, Cn-2	32.0	1.25	
	-CH_2-CH_3	all FAs, Cn-1	22.8	1.29	
	-CH_3	all FAs, Cn	14.1	0.86	
	-CH_2O-CHO-CH_2O-	G1, G1'	62.2	4.04 (α), 4.25 (β)	
	-CH_2O-CHO-CH_2O-	G2	69.1	5.18	
Δ ^{7}-sterol ester nucleus	-CH_2-	C1	37.1	1.13 (α), 1.79 (β)	[31, 32, 33]
	-CH_2-	C2	27.7	1.79 (α), 1.39 (β)	
	-CH-COO-	C3	73.1	4.62	
	-CH_2-	C4	34.1	1.69 (α), 1.28 (β)	
	-CH-	C5	40.2	1.42	
	-CH_2-	C6	29.7	*not resolved*	
	-CH=C$(CH)_2$-	C7	117.6	5.12	
	-CH=C$(CH)_2$-	C8	139.7	--	
	-CH-	C9	49.5	1.66	
	H_3C-C$(CH)_2CH_2$	C10	34.2	--	
	-CH_2-	C11	21.7	1.54 (α), 1.48 (β)	
	-CH_2-	C12	39.8	1.21 (α), 1.97 (β)	
	H_3C-C$(CH)_2CH_2$	C13	43.3	--	
	-CH-	C14	55.1	1.80	
	-CH_2-	C15	23.2	1.55 (α), 1.39 (β)	
	-CH_2-	C16	28.1	1.89 (α), 1.26 (β)	
	-CH-	C17	56.3	1.23	
	-CH_3-	C18	11.9	0.53	
	-CH_3-	C19	12.9	0.79	

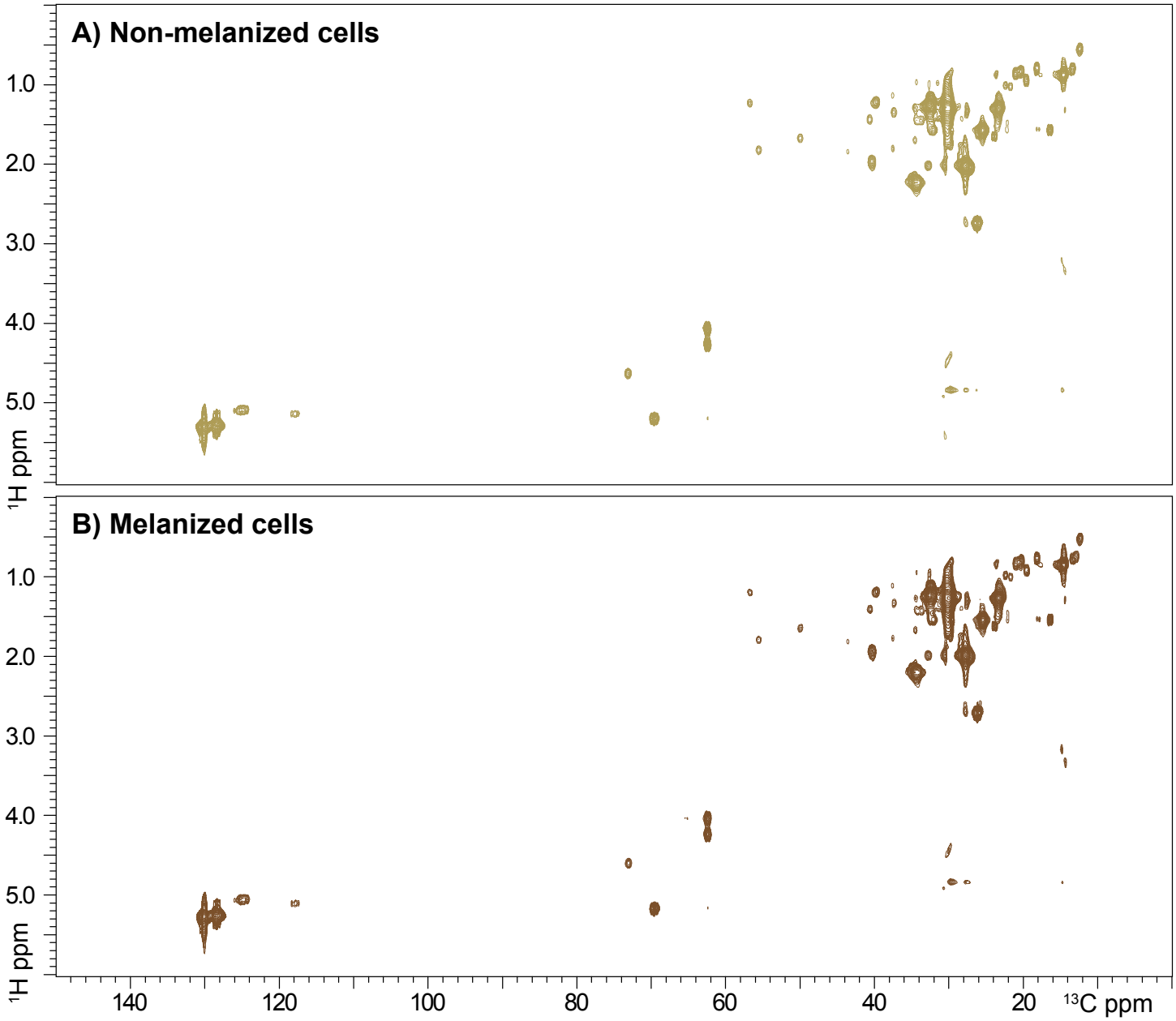
Supplementary Table 1 cont.

Constituent	Functional group	Assignment	^{13}C ppm	^{1}H ppm	References
Sterol ester side chain "A" (ergosta-7-enol)	-CH-	C20	36.9	1.33	
	-CH_3-	C21	19.1	0.92	
	-CH_2-	C22	34.2	0.93 (α), 1.49 (β)	
	-CH_2-	C23	30.9	0.93 (α), 1.37 (β)	
	-CH-	C24	39.2	1.2	[36]
	-CH_3-	C24(1)	*not observed**	--	
	-CH-	C25	31.7	1.55	
	-CH_3-	C26	20.7	0.81	
	-CH_3-	C27	18.1	0.77	
Sterol ester side chain "B" (ergosta-7,22-dienol)	-CH-	C20	40.6	1.98	
	-CH_3-	C21	21.3	1.00	
	-CH=CH-	C22	135.6	5.15	
	-CH=CH-	C23	132.0	5.20	
	-CH-	C24	43.1	1.83	[33, 34, 35]
	-CH_3-	C24(1)	*not observed**	--	
	-CH-	C25	33.3	1.44	
	-CH_3-	C26	19.9	0.81	
	-CH_3-	C27	19.9	0.81	
Polyisoprenoids	-CH_2-C(CH_3)=CH-	C1, trans	39.9	1.94	
	-CH_2-C(CH_3)=CH-	C2, trans	134.4	--	
	-C(CH_3)=CH-	C3, trans	124.4	5.07	
	-C(CH_3)=CH-CH_2-	C4, trans	27.1	2.06	
	-C(CH_3)=CH-	C5, trans	16.0	1.56	[37-40]
	-CH_2-C(CH_3)=CH-	C1, cis	32.4	1.99	
	-CH_2-C(CH_3)=CH-	C2, cis	135.0	--	
	-C(CH_3)=CH-	C3, cis	124.8	5.07	
	-C(CH_3)=CH-CH_2-	C4, cis	26.7	1.99	
	-C(CH_3)=CH-	C5, cis	23.5	1.63	

*Not observed due to the use of ^{15}N,^{12}C-glycine as the sole nitrogen source for ^{15}N-enrichment in our isotopic labeling scheme. Upon liberation of NH_4^+ via the nitrogen cleavage system, the natural abundance glycine Cα carbon is shunted into the one-carbon pool (104, 105). This pool functions as a source of methyl groups that are used for various reactions (106), including methylation at the sterol nucleus C24 position: the C24-methyltransferase enzyme responsible for this reaction is dependent on S-adenosylmethionine (SAM) for the donation of methyl groups, which are drawn from the one-carbon pool (107). Thus, the C24 methyl group is not ^{13}C-labeled, and as a result is not visible in our spectra.



Supplementary Figure 1. 1D ^{13}C quantitatively reliable DP (50-s recycle delay) spectra of wet whole melanized *C. neoformans* cells from the A) WT H99 and B) acapsular mutant Cap59 strains grown in [U-$^{13}C_6$]glucose-enriched media, demonstrating that the signals arising from capsular polysaccharides (~60-105 ppm) diminish the relative signal intensity of other constituents such as lipids. The data were acquired on cells that were never lyophilized, retaining the good spectral resolution of the relatively rigid moieties. The tallest peak in each spectrum was set to full scale. Arrows point to peaks characteristic of the O-acetyl group in glucuronoxylomannan (108, 109), the primary polysaccharide comprising the *C. neoformans* capsule, which are not observed in the Cap59 mutant strain cell sample.



Supplementary Figure 2. 2D ^{1}H-^{13}C INEPT-HETCOR spectra of A) melanized and B) non-melanized *C. neoformans* cells from the Cap59 acapsular mutant strain grown in [U-$^{13}C_6$]glucose-enriched media displaying signals that are exclusively attributable to mobile lipids. The cross-peaks observed in both whole-cell spectra are identical to one another and also to those displayed in the 2D ^{1}H-^{13}C INEPT-HETCOR spectrum of melanin ghosts (shown in Fig. 1, bottom panel). Identical cross-peaks are observed in both whole cell spectra and in the 2D ^{1}H-^{13}C INEPT-HETCOR spectrum of melanin ghosts (shown in Fig. 1, bottom panel), indicating that all three sample types contain the same predominant lipid species.