Insight into *Cryptococcus* Cell Wall, Capsule, and Melanin Using Solid-State NMR

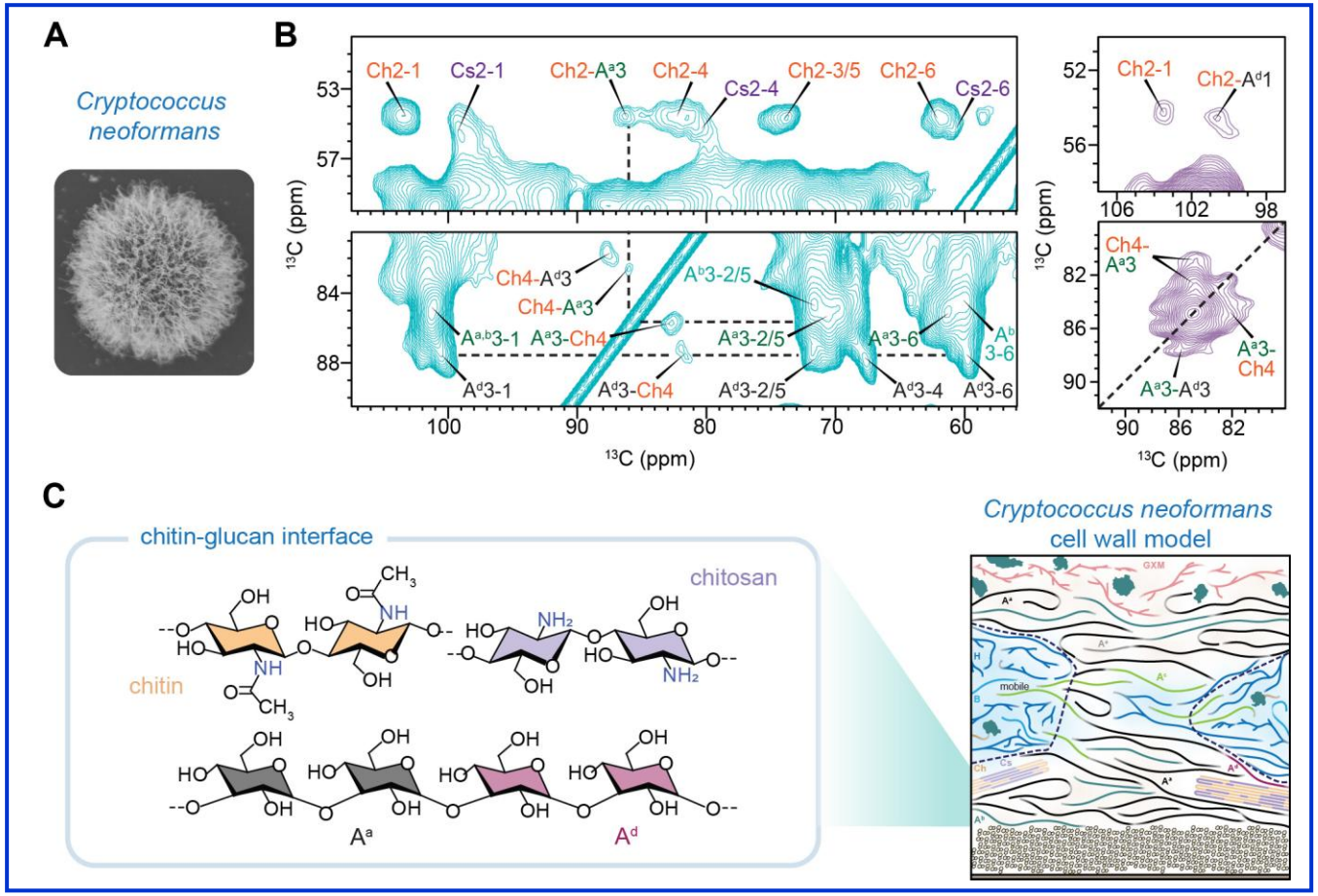
Ankur Ankur[1], Jayasubba Reddy Yarava[1], Isha Gautam[1], Faith J. Scott[2], Frederic Mentink-Vigier[2,3], Christine Chrissian[4], Li Xie[1], Dibakar Roy[1], Ruth E. Stark[4], Tamara L. Doering[5], Ping Wang[6], Tuo Wang[1]

1. Michigan State University; 2. NHMFL; 3. FSU; 4. City College of New York; 5. *Washington University*; 6. Louisiana State University

. **Funding Grants:** K. M. Amm (NSF DMR-2128556); Wang (NIH AI173270); T.L. Doering (NIH AI192892; AI175875); R.E. Stark (NIH AI171093); NHMFL (NIH GM148766)

Cryptococcus neoformans is a pathogenic fungus that cause life-threatening infections in immunocompromised individuals, driven by virulence factors such as its antiphagocytic polysaccharide capsule, a complex cell wall that supports immune evasion and stress resistance, and melanin production that protects against oxidative damage. Solid-state NMR of intact *Cryptococcus* cells **(Fig. 1A)** reveals five forms of α-1,3-glucans (A^a, A^b, A^c, A^d, and A^e) in its cell wall that structurally bridge melanin and capsule layers. These polysaccharides interact with chitin, chitosan, and β-glucans to create a molecular scaffold that underlies virulence and antifungal resistance.

Dynamic nuclear polarization (DNP) solid-state NMR enabled the detection of a previously unidentified minor α-1,3-glucan, designated type-d α-1,3-glucan (A^d), which was not observable using conventional room-temperature NMR methods. The $^{13}C/^{13}C$ spectra with 100 ms dipolar-assisted rotational resonance (DARR) mixing **(Fig. 1B, left)** and 15 ms proton assisted recoupling (PAR) mixing **(Fig. 1B, right)** demonstrate that A^d represents a minor yet highly ordered α-1,3-glucan form. These data further reveal that A^d colocalizes with type-a α-glucan (A^a), and that both glucan forms are physically associated with chitin microfibrils, collectively contributing to the structural integrity of the *Cryptococcus* cell wall. These results provide an in-depth understanding of the structural complexity of cryptococcal cell walls (**Fig. 1C**) and provide molecular insights that are essential for the development of effective antifungals against cryptococcosis.



Facilities and instrumentation used: 600 MHz/395 GHz MAS-DNP spectrometer at the National High Magnetic Field Laboratory (Tallahassee, FL, USA) **Citation:** Ankur, A.; Yarava, J.; Gautam, I.; Scott, F.; Mentink-Vigier, F.; Chrissian, C.; Xie, L.; Roy, D.; Stark, R.E.; Doering, T.L.; Wang, P.; Wang, T., *Polymorphic α-Glucans as Structural Scaffolds in Cryptococcus Cell Walls for Chitin, Capsule, and Melanin: Insights From ^{13}C and ^{1}H Solid-State NMR,* **Angewandte Chemie International Edition**, e202510409(2025) **doi.org/10.1002/anie.202510409**