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Characterization of the structure and function of self-assembling hydrophobin proteins

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Hydrophobins are low molecular weight (5-20 kDa) self-assembling proteins secreted by fungi that accumulate at hydrophobic-hydrophilic interfaces and are extremely surface-active. Hydrophobins can also undergo a structural rearrangement and oligomerize to form rodlets, which are an insoluble functional amyloid that coats fungal spores to act as a water repellent, facilitate dispersal into the air, and prevent immune recognition. Rodlets are extremely durable and due to their biochemical properties they are a target for commercial application. To better understand what protein sequence characteristics determine hydrophobin properties, we are characterizing the structure and properties of diverse class IB hydrophobins from various fungal sources. We have expressed hydrophobins in *E. coli* and purified them to homogeneity using immobilized metal ion affinity and ion exchange chromatography. We then used nuclear magnetic resonance spectroscopy to characterize the high-resolution molecular structures of hydrophobins and are comparing their self-assembly properties with thioflavin-T fluorescence assays and atomic force microscopy. These experiments will form the basis of future mutagenesis experiments to develop new hydrophobins with desired properties.

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