Structural insights into biofilm polysaccharide de-*N*-acetylation in the fungus *Aspergillus fumigatus*

Natalie Bamford[1], Francois Le Mauff[2], Hanna Ostapska[2], Don Sheppard[2], Lynne Howell[1].

[1]Department of Biochemistry, University of Toronto, Toronto, Ontario, Canada; [2]Infectious Diseases and Immunity in Global Health Program, Research Institute of the McGill University Health Centre, Montréal, QC, Canada

Aspergillus fumigatus is an opportunistic fungal pathogen that causes acute and chronic invasive infections. The exopolysaccharide galactosaminogalactan (GAG) is a virulence factor essential for *A. fumigatus* biofilm formation. GAG aids in host immune evasion, adherence to tissue and surfaces, as well as antifungal resistance. After synthesis and export of GAG across the cell membrane the secreted protein, Agd3, partially de-*N*-acetylates the *N*-acetylgalactosamine component of the polymer. Agd3 belongs to the carbohydrate esterase 4 (CE4) family and we have previously shown that deacetylation by Agd3 mediates GAG dependent virulence, rendering the polymer adherent to multiple surfaces including the fungal cell wall. Recently, we determined the structure of Agd3, recombinantly expressed in *Pichia pastoris*, to 2.79 Å using zinc single-wavelength anomalous dispersion by combining three datasets from two isomorphous crystals. The structure revealed a compact three-domain architecture not observed in previously studied CE4 enzymes. The CE4 domain has an active site groove that is elongated by the smaller N-terminal domain creating an extended cleft on one face of the enzyme. Substrate specificity and location of deacetylation in *N*-acetylgalactosamine oligomers has been investigated using MALDI-TOF mass spectrometry. Combined, the structure and our functional characterization of the protein shed light on the mechanism of GAG maturation.