Towards solving the structure of loosenin-like protein from *Phanerochaete carnosa*

Laura Okmane[a], Emma Master[b], Mats Sandgren[a], Jerry Ståhlberg[a]

[a]Department of Molecular Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden
[b]Department of Bioproducts and Biosystems, Aalto University, Helsinki, Finland; Department of Chemical Engineering and Applied Chemistry, University of Toronto, Toronto, Canada.

Loosenins (LOOS) and loosenin-like (LOOL) proteins are homologous to the N-terminal domain of expansins, which are ubiquitous in plants and responsible for the cell wall loosening process during cell growth. The LOOS and LOOL proteins lack a C-terminal Ig-like domain that is present in expansins, and they consist of a single double-Ψ β-barrel domain that is structurally related to family-45 glycosyl hydrolases (GH45). Despite having structural similarity, loosenin-like proteins lack the enzymatic activity of most GH45's, which in turn often lack the wall extension activity of expansin-like proteins.[1] The action mechanism of cell wall loosening by expansins remains unknown, but is speculated to be directed towards selectively untangling cellulose fibrils by hydrolysing certain load-bearing structures in the cell wall, rather than cleaving other easily accessible β-1,4 linkages in the cell wall. Loosenin-like genes are wide-spread among cellulolytic fungi. One loosenin from a wood-degrading fungus has been shown to cause swelling of cotton fibers and to boost the activity of cellulases on recalcitrant plant biomass material.[2]

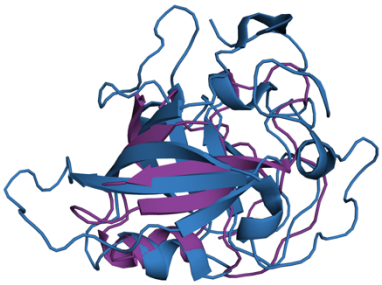

Figure 1. LOOL12 homology model superimposed on PcCel45A.

To date, no structure is available of any LOOL. In our pursuit to understand the action mechanism of expansin-like proteins, we are preparing a range of LOOL proteins for crystallization towards structure determination.

[1] C. M. Payne et al. Fungal Cellulases. *Chemical reviews* **2015**, 115 (3), 1308-1448.

[2] R.E. Quiroz-Castañeda et al. Loosenin, a novel protein with cellulose-disrupting activity from *Bjerkandera adusta*. *Microbial Cell Factories* **2011**, *10*, 8.