POSTER 10

# Novel enzymes for the analysis and glycoengineering of therapeutic glycoproteins

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Many proteins of clinical and pharmaceutical interest are *N*-glycosylated, being their bioactivity, pharmacokinetics and pharmacodynamics affected by the glycan structures they carry. Thus, one of the biggest challenges in the pharmaceutical and biomedical areas is the manufacturing of glycopeptides and glycoproteins with homogenous and defined oligosaccharide structures.
Endo-β-*N*-acetylglucosaminidases (ENGases) of the glycoside hydrolase (GH) family 85 are a class of enzymes (EC 3.2.1.96) that, in addition to hydrolytic activity against the diacetylchitobiose core of *N*-glycans, can also display transglycosylation activity. These enzymes have increasingly become a focus of interest due to their useful applications in the analysis and glycoengineering of therapeutic glycopeptides and glycoproteins.
Although family GH85 currently contains 768 members, only 11 GH85 ENGases have been characterized thus far. Envisioning the identification of new GH85 ENGases with useful action, this study focused on two new putative ENGases of this family: Q752H6 from the filamentous fungus *Ashbya gossypii* and C5DRB8 from the yeast *Zygosaccharomyces rouxii*. Previous results hinted at the existence of ENGase activity in these organisms, and therefore this work aimed at using *in silico* approaches to assess about the potential activity of their putative ENGases. Data obtained from multiple alignments and homology-based models allowed obtaining indications on possible 3D structures and catalytic residues. The closest structural homolog in the Protein Data Bank (PDB) for the putative ENGases from *A. gossypii* and *Z. rouxii* was Endo-A from *Arthrobacter protophormiae* (PDB: 2VTF.1.A). Considering the 3D models generated with SWISS-MODEL (ExPASy), only the ENGase from *Z. rouxii* presents the typical $(\beta/\alpha)_8$-TIM-barrel structure of ENGases, indicating that this protein likely presents ENGase activity. Ongoing work comprises the recombinant production, purification and characterization of this novel enzyme.

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