

## **OW9 Enrichment and characterisation of mucin-like O-linked glycopeptides**

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Heavily O-glycosylated peptides can now be characterised.

Highly glycosylated domains are present in many proteins across a wide range of species. The domains are often repeated and are characterized by a high content of serines and threonines that are heterogeneously O-glycosylated, making them very difficult to analyse. We have used a family of fungal (*Trichoderma reesei*) glucanases, that contain these domains, as a model system to develop new sample preparation and mass spectrometric methods to characterise both site occupancy and attached glycan structures. This family includes cellobiohydrolase (CBH) I and II and endoglucanase (EG) I and II.

Different proteases like trypsin, papain, proteinase K and others, were used to liberate the mucin-like domains. A range of chromatographic media such as HILIC, PGC and C18 were used to develop the best method of enrichment of these highly glycosylated domains for MS analysis. The enriched glycopeptides and their released sugars were analysed by MALDI and LC-ESI MS and CID and ETD MS/MS (Microflex™ and HCTultra, Bruker Daltonics).

The structures of the released oligosaccharides were deduced from MS, MS/MS and compositional data. Using these glycan masses, we developed a computer algorithm to more easily identify those masses which corresponded to glycopeptides in the complex “porcupine” of MS peaks. Several different approaches of ETD MS/MS fragmentation were used to try to assign the specific oligosaccharide substitution at each site of glycosylation.