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COLLOQUIUM

Hybrid HPLC-MS techniques employed to the characterization protein structure: lessons learned from the analysis of simple and highly complex glycoproteins

Speaker:

Univ Prof Mag Dr Christian Huber, Professor of Chemistry for Biosciences and head of the Bioanalytical Research Labs at the University of Salzburg, Austria.

Time:

Thursday, May 08, 2025 – 1 pm

Venue:

ISAS Campus, Lecture Hall
Otto-Hahn-Straße 6b
44227 Dortmund

&

WebEx: https://t1p.de/2ds6r
Meeting-ID: 2737 581 8938
Password: E3WwrwwdY37

Abstract

Katharina Böttinger, Christof Regl, Fiammetta di Marco, Maximilian Lebede, Gabriele Blümel, Christian C. Huber

Hybrid HPLC-MS techniques employed to the characterization protein structure: lessons learned from the analysis of simple and highly complex glycoproteins

The heterogeneity introduced by glycans associated with pharmaceutical protein drugs plays crucial roles in defining drug safety and therapeutic efficacy, and therefore, its reliable detection and quantification are essential. Challenges in qualitative and quantitative glycan analysis result mainly form the macroheterogeneity of glycosylation sites as well as from the microhereogeneity of associated glycan structures together with the combinatorial explosion of possible combinations of both. Moreover, the quantification is complicated by the fact that the quantitative information typically gained by mass spectrometry at different levels of glycoprotein structure, more specifically released glycans, glycopeptides, protein subunits, and intact protein are not always fully consistent in terms of quantitative responses.

Our study focuses on a multi-level quantification approach for glycosylation analysis in monoclonal antibodies. Mass spectrometric data is evaluated mainly employing open-source software tools. Released N-glycan and glycopeptide data form the basis for integrating information across different structural levels up to intact glycoproteins. Comprehensive comparison shows that indeed, variations across structural levels are observed especially for minor abundant species. Utilizing MoFi (short for modification finder), a tool for annotating mass spectra of intact proteins, we demonstrate quantification of isobaric glycosylation variants at the

intact protein level. Our workflow's utility is demonstrated on NISTmAb, rituximab and adalimumab, profiling their minor abundant variants for the first time across diverse structural levels. Finally, the muti-level approach is extended to highly glycosylated, heterodimeric proteins of the gonadotropin family as well as the lysosomal α-1,4-glucosidase enzyme, for which more than 42.000 protein isoforms were detectable.