**Determination of core fucosylation in glycopeptides of monoclonal antibodies**

**Methods**

The MOPC21 monoclonal antibody and α-1-Antitrypsin (Sigma) including α-1-Acid Glycoprotein were reduced and alkylated. The antibody was trypically digested. Antitrypsin sample was desalted, and subsequently digested with trypsin, Proteinase K and GluC. Peptides and glycopeptides were separated by RP-LC and fractions analyzed by ESI-ITMS (zma20Z speed ETD) and MALDI-TOF/TOF-MS (ultraflextreme) in positive ion mode (both Bruker). ProteinScape 3.0 (Bruker) was used to classify MS/MS spectra of glycopeptides with and without core fucosylation in two steps (Figure 1). Subsequent glycan database searches using the GlycoQuest search engine (Bruker) provided the glycan structures. Protein database searches were started on the same bioinformatics platform for MALDI MS/MS spectra (MassTax, Matrix Science).

**Result MALDI**

- Core fucosylation is determined via a MALDI specific pattern of 5 peaks (Wuhrer et al., 2007, Figure 2).
- This pattern includes the peptide mass of the glycopeptide.
- Both information is used by ProteinScape to fish core fucosylated glycopeptides and to determine their peptide masses, respectively.
- Subsequently, glycan database searches using GlycoQuest as search engine identified the glycan structures confirming the fucosylation state.
- Peptide database searches using the peptide mass and peptide fragmentation identified the peptide sequence.
- We applied the method to various glycopeptides.
- MOPC21 glycan structures were found to be either core- or not fucosylated (Figure 2).
- α-1-Acid Glycoprotein mainly shows fucoses at the antennae and not at the core (Figure 2). The fragmentation pattern identifies the glycan not to be core fucosylated.

**Conclusion**

- A 5 peak pattern is frequently observed for core fucosylated glycopeptides (Figure 2). It differs from the respective MALDI pattern.
- The ESI pattern includes the mass peptide plus GlcNAc.
- Both information is used by ProteinScape to extract core fucosylated glycopeptides and to determine their peptide masses, respectively.
- We applied the method to various glycopeptides.
- Core fucosylation was found on MOPC-21 N-glycan structures (Figure 2).
- Spectra of fucosylated glycopeptides of the alpha-1-Antitrypsin sample reliably contain oxonium ions 512 and 530 (B and C fragments for +1 M + 1).
- In some MS/MS spectra we observed a low intense Y fragment (< 10 % of the pattern base peak) „peptide + 18“. Since we do not have any hint for fucose migration (Wuhrer et al., 2006), we assume that a mostly antennary fucosylated glycopeptide contains trace amounts of core fucosylation.

**Bioinformatics**

- Dedicated workflow for the analysis of glycopeptides consisting of classification steps - incl. peptide mass determination - to detect:
  - core fucosylated glycopeptide spectra
  - antennary and not fucosylated glycopeptide spectra
- Glycan database searches with subsequent manual inspection of the search result, profiling from high interactivity between result tables and annotated MS/MS spectra
- Successful application to ESI and MALDI data: 19 core fucosylated and 18 not fucosylated glycopeptides were determined for both MS instruments together.