Landes Bioscience www.landesbioscience.com

Supplemental Material to:

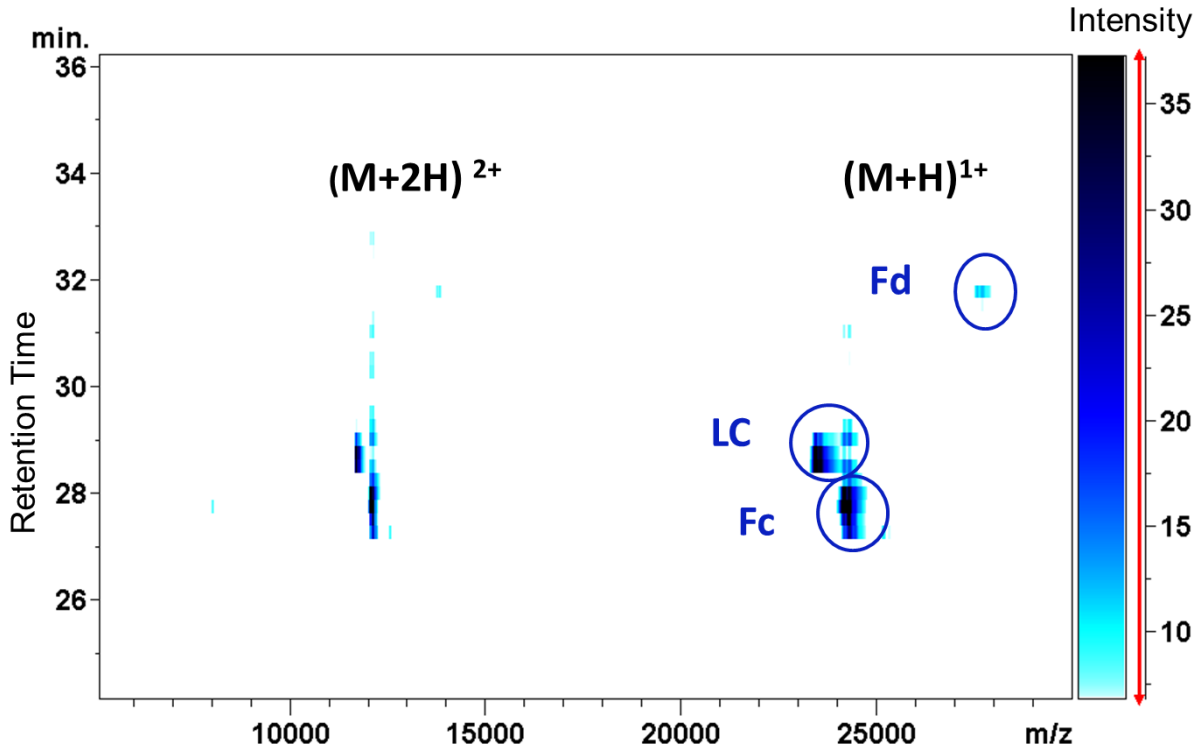
Daniel Ayoub, Wolfgang Jabs, Anja Resemann, Waltraud Evers, Catherine Evans, Laura Main, Carsten Baessmann, Elsa Wagner-Rousset, Detlev Suckau, Alain Beck

Correct primary structure assessment and extensive glyco-profiling of cetuximab by a combination of intact, middle-up, middle-down and bottom-up ESI and MALDI mass spectrometry techniques

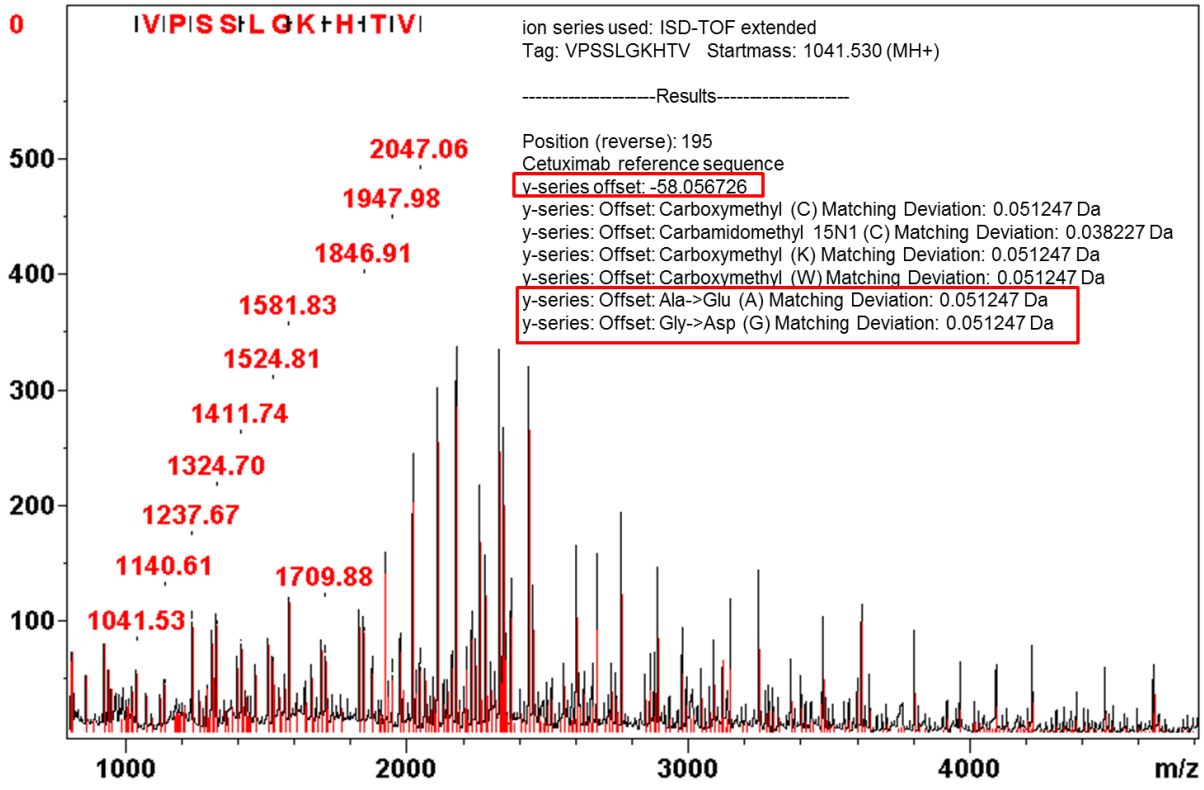
2013; 5(5)
http://dx.doi.org/10.4161/mabs.25423

http://www.landesbioscience.com/journals/mabs/article/25423/

Supplementary Figure 1: LC-MALDI-MS of Cetuximab digested with endoglycosidase F2 and IdeS. The fraction at 28.5 min contained the light chain and was used for Top-down sequencing by MALDI-ISD.

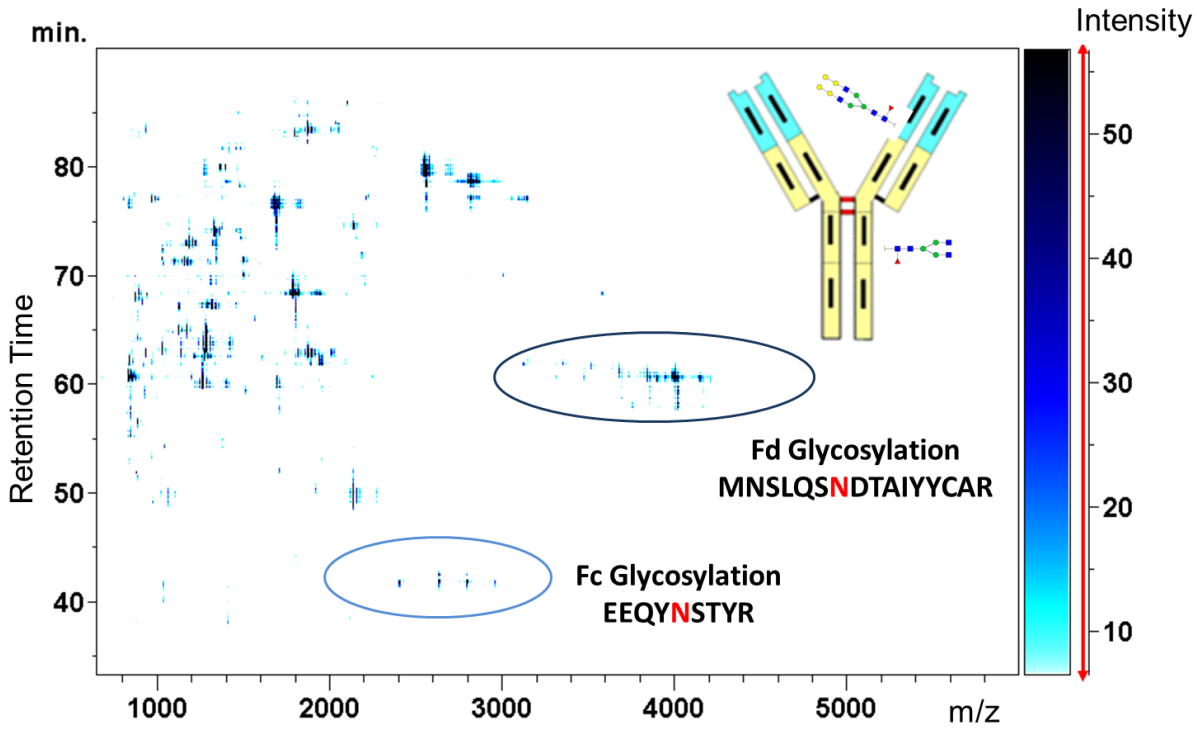


Supplementary Figure 2: A c-terminal sequence tag was generated in Biotools starting with m/z 1041.53 and compared to the cetuximab reference sequence. The C-terminal ISD fragments (in this case y-ions) were detected and a mass offset of 58 Da was found. This corresponds to the mass offset found for the intact light chain and suggest a modification at the near C-terminus of the light chain (below m/z 1041.5). Assuming a modification of 1 single amino acid, Biotools compared the mass offset of 58 Da with modifications and mutations in unimod and proposed to amino acid exchanges: Alanine to Glutamic acid and Glycin to Asparagine. The first proposal would end up in „RGEC“ as C-terminus for the Cetuximab Light chain, a common light chain sequence.

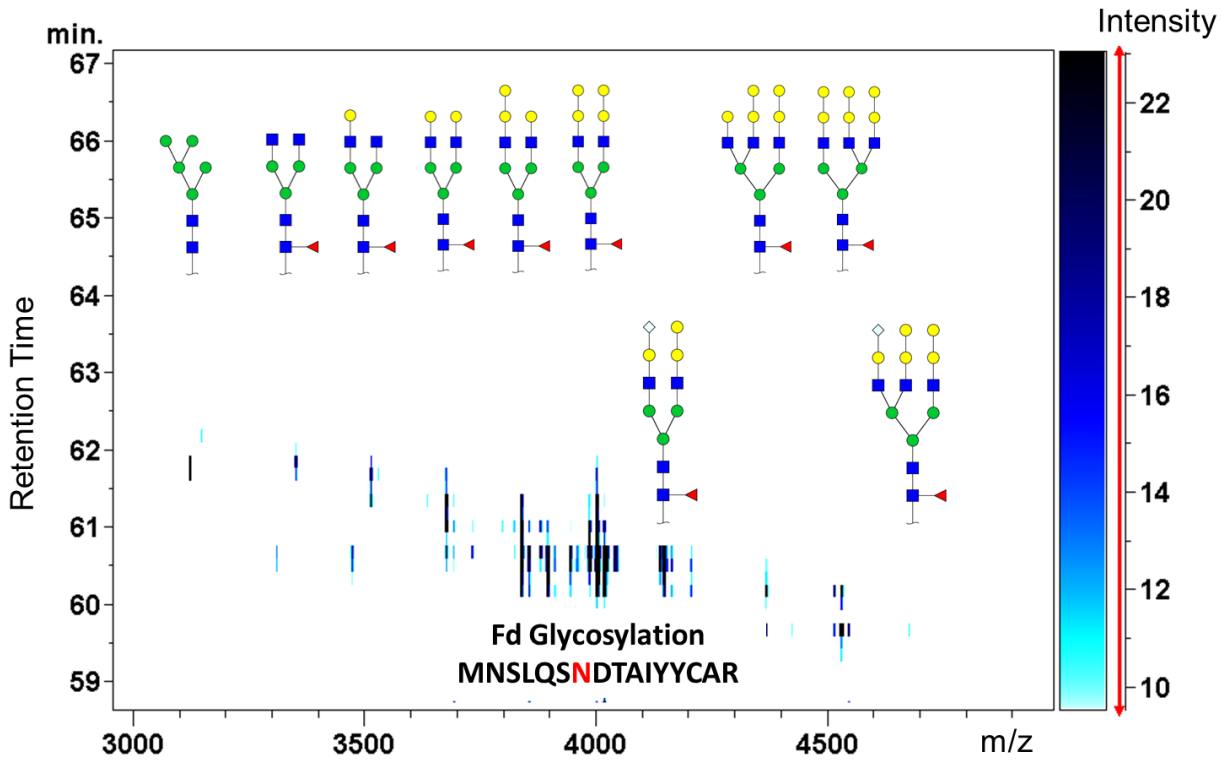


Supplementary Figure 3: Peptide Mapping: Combined maXis LC-MS/MS runs of a tryptic and a GluC digests results in 100% MS and MS/MS sequence coverage of the light chain of cetuximab. The Sequence View indicates the matching peptides (MS information) as gray bars and observed b and y-type fragment ions of the peptides (MS/MS information) as two series with red boxes within the peptide box, leading to a full characterization of the protein sequence. Every residue is validated by at least one fragment ion.

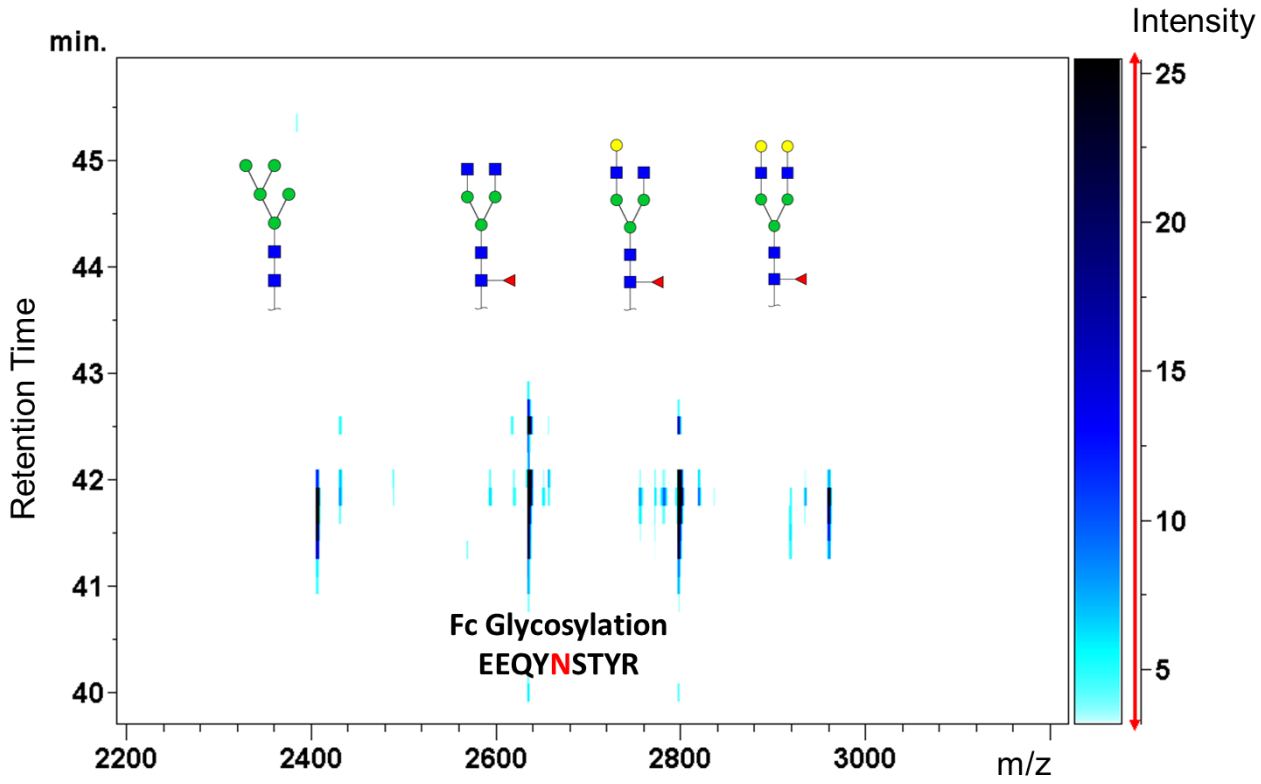
Supplementary Figure 4: LC-MALDI-TOF/TOF analysis of cetuximab tryptic digest in DHB as matrix. Survey view of m/z in relation to retention time. Glycopeptide fractions are clearly separated from the distribution of other peptides. N-linked glycopeptides from Fc region (EEQYNSTR) are highlighted by dark blue shape, the N-linked glycopeptides from Fd region (MNSLQSNDTAIYYCAR) are marked in bright blue.



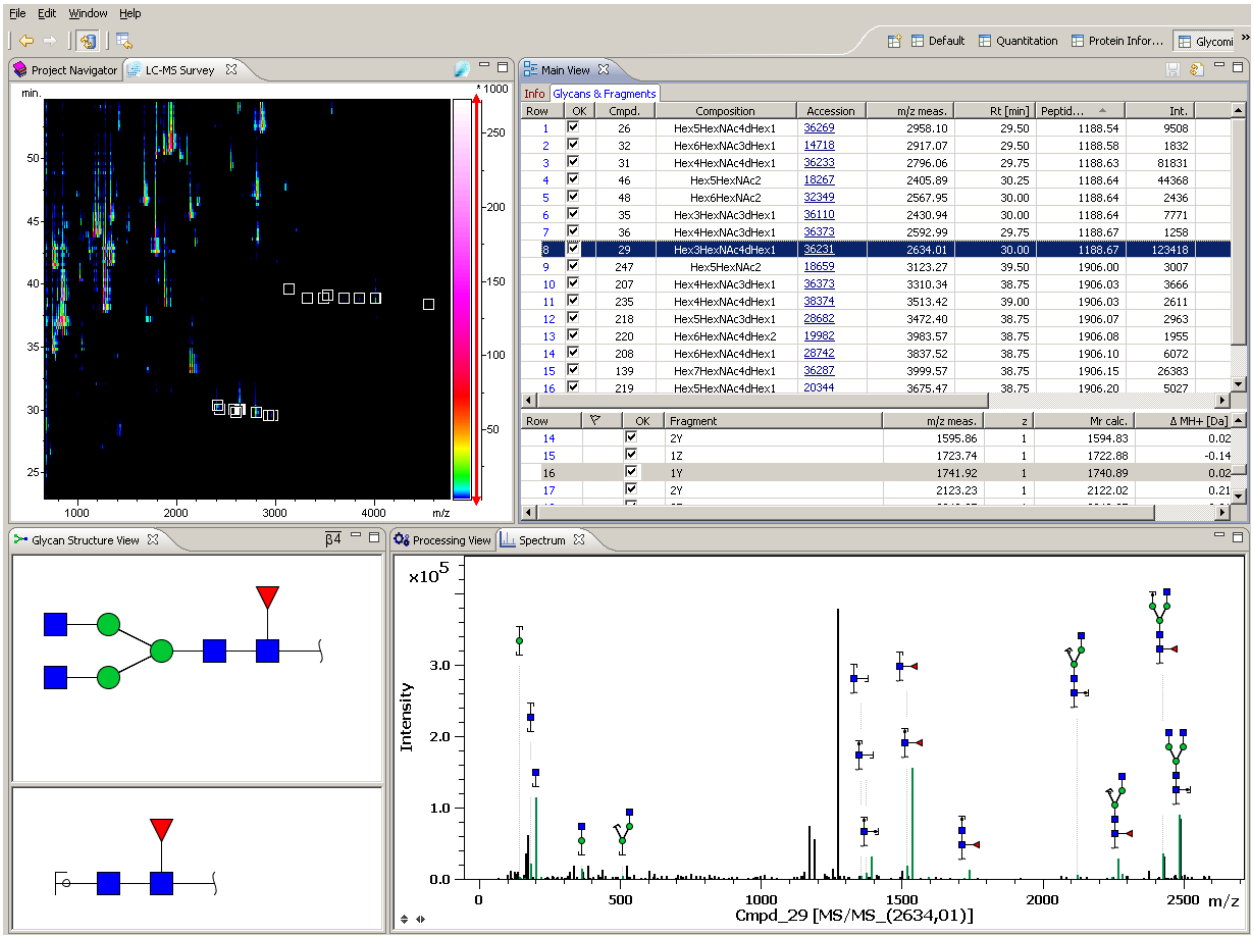
Supplementary Figure 5: LC-MALDI-TOF/TOF analysis of cetuximab tryptic digest in DHB as matrix. Survey view of m/z in relation to retention time. Glycopeptide fractions are clearly separated from the distribution of other peptides. N-linked glycopeptides from Fc region (EEQYNSTR) are highlighted by dark blue shape, the N-linked glycopeptides from Fd region (MNSLQSNDTAIYYCAR) are marked in bright blue.



Supplementary Figure 6: LC-MALDI-TOF/TOF analysis of cetuximab tryptic digest in DHB as matrix. Survey view of m/z in relation to retention time. Glycosylation analysis of Fc region (EEQYNSTR) using the same procedure as described for the Fd glycosylation.

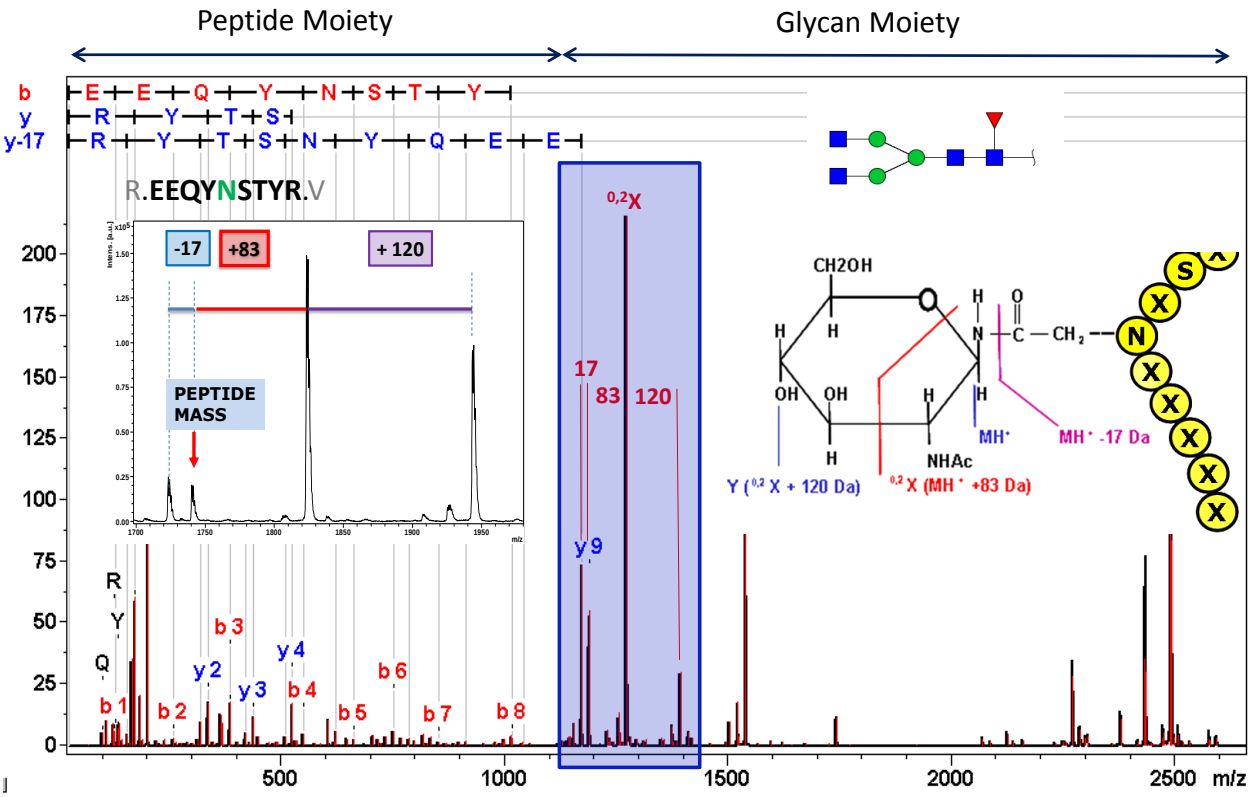


Supplementary Figure 7: LC-MALDI-MS/MS spectrum of tryptic Fc glycopeptide of most abundant glycoform. The GlycoQuest result is displayed in the graphical user interface of ProteinScape.



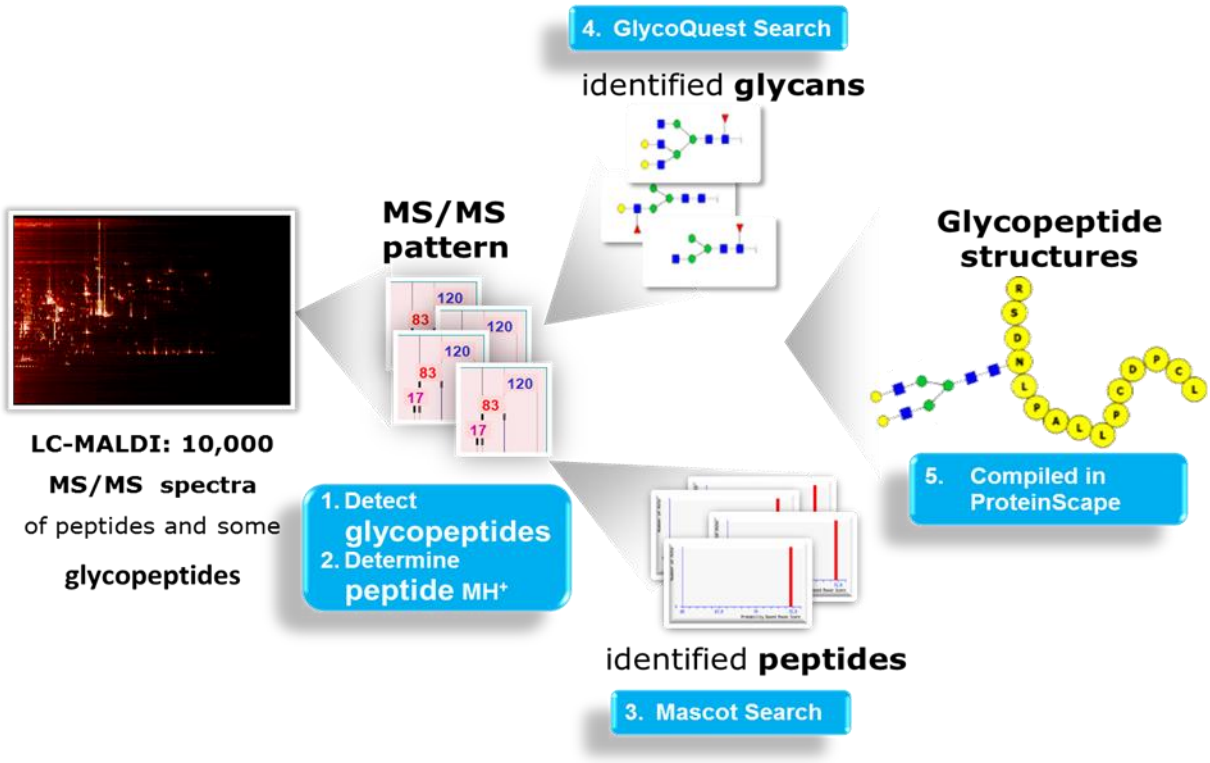
Supplementary Figure 8: Glycopeptide analysis workflow utilizing a N-glycopeptide specific MALDI-TOF/TOF fragmentation pattern (a) and a software analysis strategy (b):

a) LC-MALDI-MS/MS spectrum of tryptic Fc glycopeptide of most abundant glycoform, peptide fragments annotated. The typical MALDI N-linked glycopeptide fragmentation pattern containing the peptide mass, loss of ammonia, the N-Acetylglucosamine attached to the asparagine and the ^{0,2} X fragment of the N-Acetylglucosamine (peptide mass -17, +83 and +120 Da) used for spectrum classification and peptide MH^+ calculation are highlighted in the spectrum.

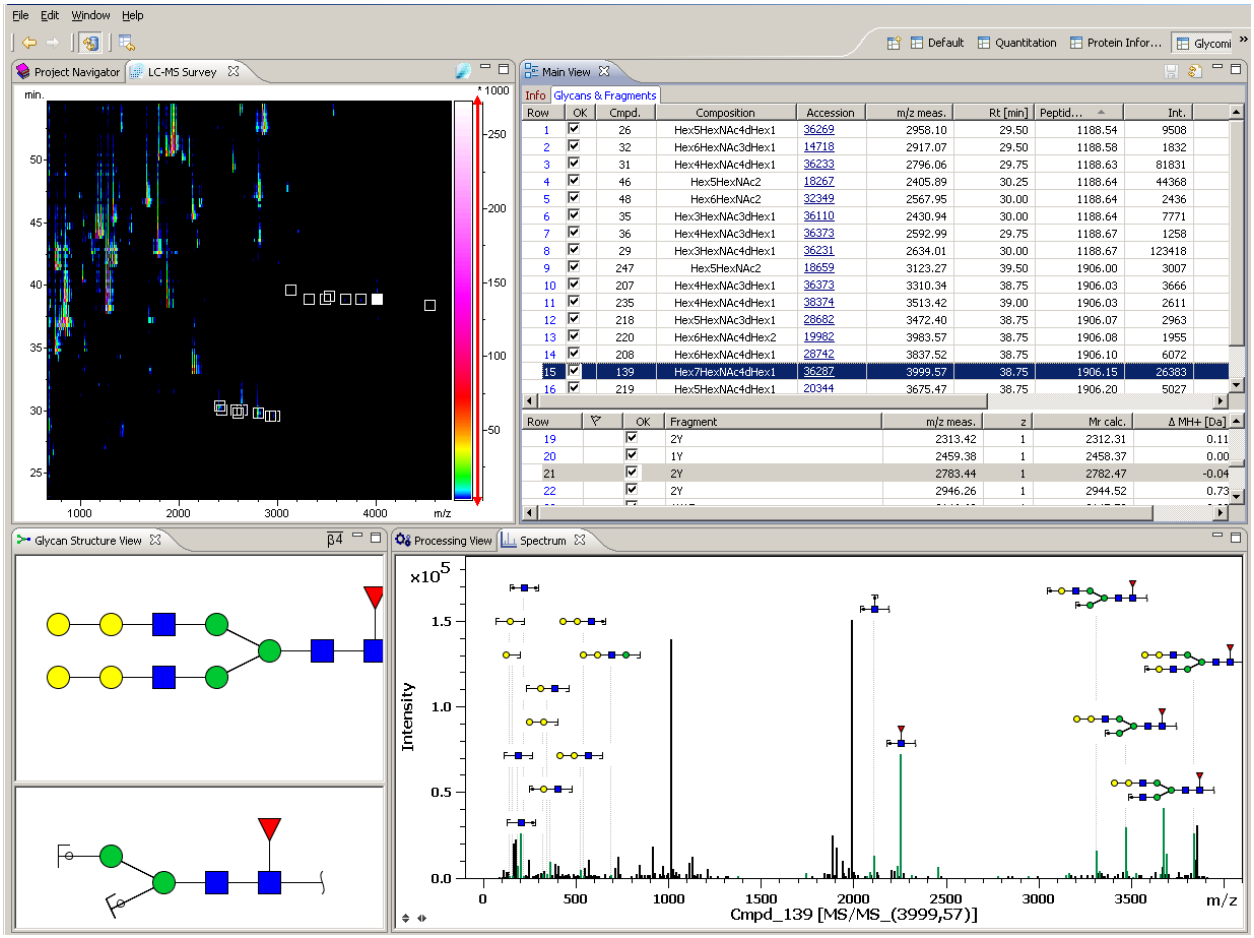


Supplementary Figure 8: Glycopeptide analysis workflow utilizing a N-glycopeptide specific MALDI-TOF/TOF fragmentation pattern (a) and a software analysis strategy (b):

b) The fragment pattern allows to 1. detect all glycopeptides (here N-glycopeptides) in the LC-MS/MS datasets and to 2. extract the aglycone, aka peptide, mass that can be used for 3. direct Mascot database searching using them as "virtual precursor ions". The identified peptide mass is subtracted from the glycopeptide MH+ mass and 4. the resulting glycan mass is used in a GlycoQuest glycan search together with all MS/MS fragments to obtain glycan structure candidates (not resolving linkage variants etc., though) . The entire analysis pipeline is implemented in ProteinScape (Bruker), which also 5. compiles the resulting glycopeptide structures.



Supplementary Figure 9: LC-MALDI-MS/MS spectrum of tryptic Fd glycopeptide with most abundant glycoforms. The GlycoQuest result is displayed in the graphical user interface of ProteinScape.



Supplementary Figure 10: LC-MALDI-MS/MS spectrum of tryptic Fd glycopeptide with most abundant glycoform showing peptide fragmentation. The peptide was identified with Mascot 2.4 using the automatically detected peptide mass 1907.1 Da.