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Technical Note

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Applicability of phenylhydrazine labelling for structural studies of fucosylated N-glycans

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ABSTRACT

Fucosylation is a common modification and its site in glycans refers to different normal and pathological processes. Despite intensive research, there is still a lack of methods to discriminate unambiguously the fucose position in one-step. In this work, we propose utility of phenylhydrazine (PHN) labeling for structural studies of fucosylated *N*-glycans by tandem MALDI mass spectrometry (MS) in the positive ion mode. PHN-tag influences the production of specific ion types and MS/MS fragmentation pattern provides useful structural information. All types of core fucosylated *N*-glycans have produced two abundant ions consistent with B- and C-glycosidic cleavages corresponding to the loss of *FucGlcNAcPHN* residue with mass 457 and 441 Da from the parent ions. These types of fragment ions in *N*-glycans without a core fucose were associated with the loss of *GlcNAcPHN* unit (311 and 295 Da) and fucose cleavage followed loss of the chitobiose residue. Since diagnostic useful cleavages produce peaks with significant intensities, this approach is also beneficial for rapid recognition of antenna from core fucosylation in glycans detected with low abundances. Moreover, in multifucosylated glycans this type of labeling allows to distinguish how many fucose residues are on the specific antenna and provides additional information on the topology of *N*-glycans such as type of antennarity or identification of bisecting moiety. The practical applicability of the approach is demonstrated on the analysis of multifucosylated *N*-glycans detected with lower abundances in lung cancer samples.

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INTRODUCTION

N-linked protein glycosylation is a venerable posttranslational modification and one of the pervasive regulatory mechanisms participated in multiply biological processes.[1] Among distinctive features of *N*-glycans is their structural variability.[2] Fucosylation is one of the structural features of glycosylated proteins.[3] The position of the fucose residue/s in the glycans results on expression of fucosylation-related enzymes, of which the up or downregulation depends on different normal and pathological processes.[4-10]

Methods for determination of this intriguing monosaccharide residue are based on using the high-performance liquid chromatography or MS after repeated digestions with specific enzymes.[11–13] MS is one the most sensitive, rapid and routinely used technique for analysis of glycoconjugates. However, it is not very useful for direct assignment of a fucose position in native glycans because of its lability and extensive migration during tandem MS experiments. Derivatization techniques minimize the fucose loss,[11,14,15] but still lead to ambiguity and are laborious for routine application.[16] To simplify and apply only MS strategy for validation of core fucosylastion, *Nwosu et al*. proposed an alternative method based on detection of *N*-glycans labeled with procainamide.[16] The core fucosylated glycans were confirmed by diagnostic peak detected at *m/z* 587 corresponding to composition *GlcNAcFucProcainamideH*$^{+}$. Whereas its absence in the spectra indicated the presence of fucose on an antenna.[16] The researcher presented useful application of this method towards verification of core fucosylation in a therapeutic protein IgG1. *Mancera-Arteua et al*. proposed μZIC-HILIC-MS/MS method in negative ion mode .[17] The absence of the Y_1 ion at *m/z* 297 in the tandem mass spectra was associated with core fucosylation. Whereas this peak was detected in the spectra of the non-fucosylated glycans. The other combined ions such as $^{2,4}A_5/Y_5$ suggested that the fucose was

not located on any antenna. Lastly, *Harvey & Struwe* demonstrated the ability of negative collision-induced dissociation combined with ion mobility for more detailed characterization of fucosylated *N*-glycans including tri-antennary ones.[18] The spectra provided useful information about number and position of fucose residues on specific antenna.

We previously reported MS analyses of *N*-glycans obtained from different sample sources by employing non-reducing type of derivatization with PHN on reducing termini of glycans.[19,20] Besides simplicity of this labeling technique, we showed on a number of different *N*-glycans that under tandem MS conditions these derivatives fragmented in a predictable manner. They preserved the advantages of important diagnostic ions observed on native glycans,[21] but avoiding ambiguity in structural assignments.[19] Here we applied this approach to demonstrate its capability for an efficient discrimination of core from antenna fucosylation. Thanks to the presence of diagnostic ions with significant intensities, this approach is applicable for a recognition of the fucose residue also in low abundant *N*-glycans. The usefulness of this rapid method is demonstrated on the analysis of larger than three antennary *N*-glycans carrying multiply fucose residues.

EXPERIMENTAL SECTION

Materials.

All chemicals, solvents, enzymes and monoclonal antibody (mab) Rituximab were purchased from Sigma. Lung cancer cells (HTB-171) were used from previous study[22] and lung cancer tissue was obtained through bronchoscopy following the protocol approved by the regional Ethics Committee of University Hospital in Brno.

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Release of *N*-glycans.

***N-glycans release from IgG1*.** Rituximab (50 μg) dissolved in 5mM of ammonium bicarbonate (AB) solution (100 μL) was incubated with PNGase F at 37°C for 1-3h. After that 2 μL was taken from a digest, spotted on the MALDI target and after derivatization analyzed as described in the MS section. ***N-glycans release from cancer samples*.** Prior to the PNGase F deglycosylation, cancer cells or biopsy samples underwent extraction as described previously.[22] Briefly, 300 μL of mixture consisting from chloroform-methanol-water (CMW in ratio 8:4:1) was added to the each sample and sonicated for 5 min at room temperature. After short centrifugation, the organic solvent was pipetted out and crude sediment was vacuum evaporated. *N*-glycans were released from samples with PNGase F in 5 mM AB solution (100 μL) at 37°C for 3h; and alternatively followed the incubation (2h) with neuraminidase (*Clostridium perfringens*). After incubation and brief centrifugation, the supernatants were fractionated on nonporous graphitized carbon cartridges. The glycan fractions were dried under vacuum.

PHN derivatization and MS analysis.

Purified glycans were reconstituted in deionized water (20 μL) and subjected to on-target derivatization.[22] Matrix was prepared by mixing of 2-aza-2-thiothymine and phenylhydrazine hydrochloride (ATT/PHN.HCl, 2:1) with the final concentration 2% in ACN/deionized water (1:1). 0.8 μL of the matrix solution was spotted on the surface of the AnchorChip target and immediately followed addition of glycan solution (~1.5 μL). To the still wet spot, 0.6 μL of PHN reagent (PHN:ACN:Water in ratio 1:0.5:4) was added and left air dry (5-10 min). MALDI-TOF/TOF-MS analysis was carried on UltrafleXtreme*TM* mass spectrometer furnished with a Smartbeam-II laser and LIFT technology (Bruker, Germany). The instrument was used in the reflectron positive mode,

calibrated externally using mixture of peptides and then internally using known glycans. Individual parent ions were selected for LIFT experiments. MS/MS spectra were evaluated manually using fragmentation rules described earlier.[19,20] For drawing glycan structures, the symbolic nomenclature recommended by the Consortium for Functional Glycomics was applied (http://www.functionalglycomics.org/). Fragment ions in tandem mass spectra were assigned using the *Domon & Costello* nomenclature.[23]

RESULTS AND DISCUSSION

For this study, *N*-glycans were obtained after enzymatic digestion from the IgG1, more complex fucosylated *N*-glycans characterized using exoglycosidase cleavages were from previous work[22] and from cancerous lung tissue obtained during bronchoscopy. In case of IgG1, *N*-glycans were analyzed immediately after digestion without applying any purification step.

Core fucosylated N-glycans. Therapeutic IgG1 (Rituximab) was chosen here as standard since this glycoprotein has simple glycan profile of known structural compositions and majority of them are core fucosylated. As reported previously derivatization with PHN is a simple reaction and in case of analysis of not very complex samples, it can provide benefit in immediate analysis of *N*-glycans without using any purification step.[20] We already illustrated this advantage on the analysis of therapeutic Herceptin.[24] Similarly, here, after 2 hours of digestion, the main glycan profile of IgG1 corresponded to that when applying HILIC purification and analysis using ultra performance liquid chromatography coupled with MS.[16] The main glycans with PHN-tag were observed at *m/z* 1575.5 ($GlcNAc_2Man_3GlcNAc_2Fuc$), 1737.6 ($Gal_1GlcNAc_2Man_3GlcNAc_2Fuc$) and 1899.7 ($Gal_2GlcNAc_2Man_3GlcNAc_2Fuc$). Under tandem MS, these peaks produced characteristic ions confirming only core type of fucosylation. In the Figure 1 are shown tandem mass spectra recorded

for biantennary complex *N*-glycans. When no fucose is on the chitobiose core, the abundant fragment ions associated with B- and C-glycosidic cleavages at highest *m/z* values are associated with the loss of 311 Da (B-ion) and 295 Da (C-ion) corresponding to the loss of *GlcNAcPHN* residue from the parent ions (Figure 1A). However, when glycan is core fucosylated, the same types of cleavages are associated with the loss of *FucGlcNAcPHN* producing abundant B,C-fragments with the typical loss of 457 Da (B-ion) and 441 Da (C-ion) from the precursors ions (Figure 1B). In this spectrum, no additional fragments corresponding to the loss of 311 and 295 Da (*GlcNAcPHN* residue) from the precursor ions were detected. Thus, confirming glycan with fucose attached only to the core region. In both MS/MS spectra shown in the Figure 1A and B, after the first glycosidic cleavages follows the abundant loss of a second GlcNAc residue from the chitobiose core. The absence of the chitobiose core mostly produces peak with higher intensity for C ion over B ions. The loss of monosaccharide residues from the antenna attached to the 3-position of the branching mannose produced abundant $B_4/Y_{3\alpha}$ ion (D-ion) at *m/z* 712 accompanied with characteristic loss of water *(m/z* 694*)* as described previously.[19–21] When analyzing the same glycan in the native form, abundant ions corresponded to the loss of monosaccharide residues from both reducing and non-reducing ends of glycan (Figure S1). At least two peaks with high intensities (*m/z* 1663 and 1298) were associated with the loss of fucose residue. These and other competing losses of monosaccharide residues did not provide opportunity to assign a core fucosylation with no ambiguity as it was possible for this glycan when labeled with PHN (Figure 1B). The similar pattern was observed in the spectra recorded for the precursor ions at *m/z* 1575, 1737 (Figure S2). In case of procainamide derivatized glycans, core fucosylation was confirmed based on the presence of the peak at *m/z* 587, frequently detected with low intensity.[16] Therefore, it was not possible to confirm this fragment in case of low abundant glycans as the authors indicated in their work. Whereas, when applying PHN labelling, the key cleavages produce peaks

with significant intensities. Thus, it provides opportunity to identify unambiguously the core fucose even in case of low abundant glycans detected at *m/z* 1372 and 1534 in IgG1 (Figure S3).

Core versus antenna fucosylation. The simple example how to validate assignment of fucose on the reducing termini discussed above and its discrimination from antenna fucosylation, is illustrated on a comparison of tandem MS spectra shown in Figure 1B and 1C. Figure 1C represents spectrum recorded from cancerous lung tissue for the same precursor ions as detected in IgG1 (Figure 1B). The peak at *m/z* 1899 analyzed in the biopsy provided fragmentation pattern corresponding to the core fucosylated glycan (Structure 1) as observed in IgG1 (Figure 1B). An additional fragments identified at *m/z* 1598 (B_5 ion) and 1604 (C_5 ion) were associated with the loss of non-fucosylated *GlcNAcPHN* residue (311 or 295 Da) and then the total loss of chitobiose core at *m/z* 1385 and 1401 (B_4 and C_4 ions). These fragments supported the presence of second isomer with no core fucose, however having this subunit on one of the antennae (Figure 1C; Structure 2). The relative summed intensities of B5 and C5 ions associated with the loss of *FucGlcNAcPHN* residue compared with the summation of the intensities of the same ion types, however associated with the loss of *GlcNAcPHN* from the parent ion, indicates that the core and antenna fucosylated glycans in the mixture are approximately in the ratio of 3:1 (Figure 1C).

Applicability of the method for identification of more fucosylated N-glycans. MS/MS patterns of PHN labeled glycans is very similar in case of the biantennaery core fucosylated glycans with bisecting moiety and carrying additional fucose residues on the arms (Figure 2). In the spectra of both glycans, any fragment ions corresponding to the reducing termini without fucose (the loss of 311 and 295 Da) was not observed. *N*-glycan with composition $Gal_2GlcNAc_3Man_3GlcNAc_2Fuc$ detected at *m/z* 2103, in its MS/MS spectrum after the loss of *FucGlcNAcPHN* residue (*m/z* 1645 and 1661) produced other dominant ion consistent with C-type of glycosidic cleavage accompanied with smaller

peak at *m/z* 1442 (B_4 ion), associated with the loss of chitobiose core (Figure 2A). The loss of monosaccharide residues from the 3-antenna was associated with a higher peak at *m/z* 915 ($B_4/Y_{3\alpha}$ ion). The presence of a bisecting moiety was recognized based on the significant loss of 221 Da from $B_4/Y_{3\alpha}$ fragment ions,[19,20] the same phenomenon as observed in MS/MS spectra of native glycans.[21] The similar principle of fragmentation we observed in the spectrum recorded for the other glycan with additional two fucoses - $Fuc_2Gal_2GlcNAc_3Man_3GlcNAc_2Fuc$, detected at *m/z* 2395 in cancer cells (Figure 2B). The fragments at *m/z* 1588 and 1604 were associated with the loss of fucose (146 Da) from B_4 or C_4 ions. The abundant fragment at *m/z* 1061 ($B_4/Y_{3\alpha}$ ion) was consistent with composition $Fuc_1Gal_1GlcNAc_2Man_2$ indicating third fucose residue linked on the 6-antenna.

The practical applicability is next presented on the analysis of more antennary multifucosylated oligosaccharides shown in Figure 3. Although both glycans were detected with low abundances, their spectra exhibited characteristic cleavages and still sufficient data to confirm presence of dominant core fucosylated structures (the loss of 311 and 295 from both precursor ions was not observed). As reported previously, fucose is very labile and there are difficulties to confirm this residue on the antenna versus core by using only tandem mass technology in the positive mode.[18] However, our results indicate that the PHN-tag on the reducing end has positive impact on fragmentation and can be used to discriminate reliably core from antennae fucosylation. Moreover, characteristic ions make possible to predict a position of this residue on the antenna type. For example, the tetra-antennary glycan with precursor at *m/z* 2922 after the total loss of fucosylated chitobiose core (B_4 or C_4 ions) and monosaccharide residues from the 3-antenna at *m/z* 1368 ($B_4/Y_{3\beta}$ ion) indicated major isomeric structure with extra two fucose residues only on the 6-antenna (Figure 3A). A similar MS/MS pattern was observed for the core fucosylated glycans detected at *m/z* 3579, carrying additional *FucGalGlcNAc* residue on the 6-arm and fifth fucose attached to one of the monosaccharide residues

on the 3-antenna (Figure 3B). The significant $B_4/Y_{3\alpha}$ peak (D-ion) appeared at *m/z* 1880 and was in a favor of main isomer with additional one fucose on the 3-antennae and three fucoses on the 6-antenna. Although, the purpose of this study was not exact assignment on which monosaccharide residue of the antenna is fucose linked, very low abundant peaks observed at *m/z* 899, 1045, 1248 and 2392 could indicate the linkage of fucose to GlcNAc residues.

Core 1,3- versus core 1,6-linked fucose. Tandem MS of different types of core fucosylated glycans from mammals (1,6-linkage) with no fucose on the antenna, did not show the presence of any fragment corresponding solely to the loss of fucose (146 Da) from the parent ions (e.g. Figure 1B, 2A, Figures S2 and 3). Instead, this loss was observed together with A-type cross-ring cleavage from the reducing end ($^{0,1}A_x$-146). On the other side, antenna fucosylated glycans, produced a peak associated with the loss of fucose residue from the parent ions. The same loss was observed in the analysis of *N*-glycans obtained from plant sources when fucose was 1,3-linked to the core region.[25] These differences are illustrated on the spectra recorded for simple *N*-glycans of the same compositions when analyzed in lung tissue with 1,6-linked core fucose (Figure S4A) and in patatins with 1,3-linked core fucose (Figure S4B). In the second spectrum, the fragment at *m/z* 861 is associated with a loss of 146 Da. However, there was not detected any ion corresponding to the loss of 311 and 295 Da (*GlcNAcPHN)* from the precursor ion in this or other analyzed plant glycans. This information indicates additional potential of this approach for rapid discrimination of 1,6- from 1,3-linked core fucose.

CONCLUSION

In this study, we demonstrate the capability of PHN-labeling for rapid recognition of fucosylation in *N*-glycans using MALDI-MS/MS analysis. Besides already proved simplicity of this procedure, we

show that this labeling provides fragmentation patterns allowing for discrimination of core versus antennae fucosylationin in one-step. Since the diagnostic useful peaks are produced with significant intensities, the method is efficient for very low abundant glycans as well. Additionally, the processing time starting with digestion and ending with MS analysis including manual data interpretation does not exceed 4 hours in case of simple samples such as biopharmaceutical relevant antibodies. The results also point to the utility of PHN-tag for differentiation of the core fucose with 1,6-linkage from that linked to 1,3-core region. Moreover, as evidenced from analysis of different fucosylated glycans from bi to five antennary structures, tandem mass spectra provide direct information on the antennarity type and presence of bisecting GlcNAc residue with additional possibility to predict how many fucoses are on a specific antenna.

ASSOCIATED CONTENT

Supporting information

Additional supporting figures as noted in the text are available free of charge on the ACS publication website at http://pubs.acs.org.

MALDI-MS/MS spectra recorded for native and PHN derivatized core fucosylated *N*-glycans with different compositions and core fucosylated *N*-glycans with the same composition identified in different samples.

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Competing financial interest

The authors declare no competing financial interest

REFERENCES

(1) Schwarz, F.; Aebi, M. Mechanisms and Principles of N-Linked Protein Glycosylation. *Curr. Opin. Struc. Biol.* **2011**, *21* (5), 576–582.

(2) Taniguchi, N.; Kizuka, Y. Chapter Two - Glycans and Cancer: Role of N-Glycans in Cancer Biomarker, Progression and Metastasis, and Therapeutics. In *Advances in Cancer Research*; Drake, R. R., Ball, L. E., Eds.; Glycosylation and Cancer; Academic Press, 2015; Vol. 126, pp 11–51.

(3) Schneider, M.; Al-Shareffi, E.; Haltiwanger, R. S. Biological Functions of Fucose in Mammals. *Glycobiology* **2017**, *27* (7), 601–618.

(4) Awan, B.; Turkov, D.; Schumacher, C.; Jacobo, A.; McEnerney, A.; Ramsey, A.; Xu, G.; Park, D.; Kalomoiris, S.; Yao, W.; Jao, L.E.; Allende, M.L.; Lebrilla, C.B.; Fierro, F.A. FGF2 Induces Migration of Human Bone Marrow Stromal Cells by Increasing Core Fucosylations on N-Glycans of Integrins. *Stem Cell Rep.* **2018**, *11* (2), 325–333.

(5) Sakae, Y.; Satoh, T.; Yagi, H.; Yanaka, S.; Yamaguchi, T.; Isoda, Y.; Iida, S.; Okamoto, Y.; Kato, K. Conformational Effects of N-Glycan Core Fucosylation of Immunoglobulin G Fc Region on Its Interaction with Fcγ Receptor IIIa. *Sci. Rep.* **2017**, *7* (1), 13780.

(6) Holst, S.; Deuss, A. J. M.; van Pelt, G. W.; van Vliet, S. J.; Garcia-Vallejo, J. J.; Koeleman, C. A. M.; Deelder, A. M.; Mesker, W. E.; Tollenaar, R. A.; Rombouts, Y.; Wuhrer, M. N-Glycosylation Profiling of Colorectal Cancer Cell Lines Reveals Association of Fucosylation with Differentiation and Caudal Type Homebox 1 (CDX1)/Villin MRNA Expression. *Mol Cell Proteomics* **2016**, *15* (1), 124–140.

(7) Pompach, P.; Ashline, D. J.; Brnakova, Z.; Benicky, J.; Sanda, M.; Goldman, R. Protein and Site Specificity of Fucosylation in Liver-Secreted Glycoproteins. *J Proteome Res* **2014**,

(8) Singh, S.; Pal, K.; Yadav, J.; Tang, H.; Partyka, K.; Kletter, D.; Hsueh, P.; Ensink, E.; KC, B.; Hostetter, G.; Xu, H.E; Bern, M.; Smith, D.F.; Mehta, A.S.; Brand, R.; Melcher, K.; Haab, B.B. Upregulation of Glycans Containing 3′ Fucose in a Subset of Pancreatic Cancers Uncovered Using Fusion-Tagged Lectins. *J. Proteome Res.* **2015**, *14* (6), 2594–2605.

(9) Li, W.; Yu, R.; Ma, B.; Yang, Y.; Jiao, X.; Liu, Y.; Cao, H.; Dong, W.; Liu, L.; Ma, K.; Fukuda, T.; Liu, Q.; Ma, T.; Wang, Z.; Gu, J.; Zhang, J.; Taniguchi, N. Core Fucosylation of IgG B Cell Receptor Is Required for Antigen Recognition and Antibody Production. *J. Immunol.* **2015**, *194* (6), 2596–2606.

(10) Zhao, Y.P.; Xu, X.Y.; Fang, M.; Wang, H.; You, Q.; Yi, C.H.; Ji, J.; Gu, X.; Zhou, P.T.; Cheng, C.; Gao, C.F. Decreased Core-Fucosylation Contributes to Malignancy in Gastric Cancer. *PLOS ONE* **2014**, *9* (4), e94536.

(11) Haslam, S. M.; Coles, G. C.; Morris, H. R.; Dell, A. Structural Characterization of the N-Glycans of *Dictyocaulus viviparus*: Discovery of the Lewis^X Structure in a Nematode. *Glycobiology* **2000**, *10* (2), 223–229. https://doi.org/10.1093/glycob/10.2.223.

(12) Jensen, P. H.; Karlsson, N. G.; Kolarich, D.; Packer, N. H. Structural Analysis of N- and O-Glycans Released from Glycoproteins. *Nature Protocols* **2012**, *7*, 1299.

(13) Royle, L.; Radcliffe, C. M.; Dwek, R. A.; Rudd, P. M. Detailed Structural Analysis of N-Glycans Released From Glycoproteins in SDS-PAGE Gel Bands Using HPLC Combined With Exoglycosidase Array Digestions. In *Glycobiology Protocols*; Brockhausen, I., Ed.; Methods in Molecular Biology; Humana Press: Totowa, NJ, 2007; pp 125–143.

(14) Pang, P.-C.; Chiu, P. C. N.; Lee, C.-L.; Chang, L.-Y.; Panico, M.; Morris, H. R.; Haslam, S. M.; Khoo, K.-H.; Clark, G. F.; Yeung, W. S. B.; Dell, A. Human Sperm Binding Is Mediated by the Sialyl-Lewis^X Oligosaccharide on the Zona Pellucida. *Science* **2011**, *333* (6050), 1761–1764.

(15) Klapoetke, S.; Zhang, J.; Becht, S.; Gu, X.; Ding, X. The Evaluation of a Novel Approach for the Profiling and Identification of N-Linked Glycan with a Procainamide Tag by HPLC with Fluorescent and Mass Spectrometric Detection. *J. Pharm. Biomed. Anal.* **2010**, *53* (3), 315–324.

(16) Nwosu, C.; Yau, H. K.; Becht, S. Assignment of Core versus Antenna Fucosylation Types in Protein N-Glycosylation via Procainamide Labeling and Tandem Mass Spectrometry. *Anal. Chem.* **2015**, *87* (12), 5905–5913.

(17) Mancera-Arteu, M.; Giménez, E.; Barbosa, J.; Peracaula, R.; Sanz-Nebot, V. Zwitterionic-Hydrophilic Interaction Capillary Liquid Chromatography Coupled to Tandem Mass Spectrometry for the Characterization of Human Alpha-Acid-Glycoprotein N-Glycan Isomers. *Anal. Chim. Acta* **2017**, *991*, 76–88.

(18) Harvey, D. J.; Struwe, W. B. Structural Studies of Fucosylated N-Glycans by Ion Mobility Mass Spectrometry and Collision-Induced Fragmentation of Negative Ions. *J. Am. Soc. Mass Spectrom.* **2018**, *29* (6), 1179–1193.

(19) Lattova, E.; Perreault, H.; Krokhin, O. Matrix-Assisted Laser Desorption/Ionization Tandem Mass Spectrometry and Post-Source Decay Fragmentation Study of Phenylhydrazones of N-Linked Oligosaccharides from Ovalbumin. *J. Am. Soc. Mass Spectrom.* **2004**, *15* (5), 725–735.

(20) Lattová, E.; Perreault, H. The Usefulness of Hydrazine Derivatives for Mass Spectrometric Analysis of Carbohydrates. *Mass Spectrom Rev* **2013**, *32* (5), 366–385.

(21) Harvey, D. J. Postsource Decay Fragmentation of N-Linked Carbohydrates from Ovalbumin and Related Glycoproteins. *J. Am. Soc. Mass Spectrom.* **2000**, *11* (6), 572–577.

(22) Lattová, E.; Bryant, J.; Skřičková, J.; Zdráhal, Z.; Popovič, M. Efficient Procedure for N-Glycan Analyses and Detection of Endo H-Like Activity in Human Tumor Specimens. *J. Proteome Res.* **2016**, *15* (8), 2777–2786.

(23) Domon, B.; Costello, C. A Systematic Nomenclature for Carbohydrate Fragmentations in FAB-MS/MS Spectra of Glycoconjugates. *Glycoconj. J* **1988**, *5*, 397–409.
(24) Perreault, H.; Lattová, E.; Šagi, D.; Peter-Katalinic, J. MALDI-MS of Glycans and Glycoconjugates. In *MALDI MS*; John Wiley & Sons, Ltd, 2013; pp 239–271.
(25) Lattová, E.; Brabcová, A.; Bartová, V.; Potěšil, D.; Bárta, J.; Zdráhal, Z. N-Glycome Profiling of Patatins from Different Potato Species of Solanum Genus. *J. Agric. Food Chem.* **2015**, *63* (12), 3243–3250.

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FIGURE LEGENDS

Figure 1. MALDI-TOF/TOF tandem mass spectra recorded for the complex biantennary *N*-glycans with precursor ions at: (**A**) *m/z* 1753.6 identified in lung tissue; (**B**) *m/z* 1899.7 detected in IgG1; and (**C**) *m/z* 1899.7 detected in biopsy of patient with lung metastases. Key symbols: ▲ *Fuc*; ■ *GlcNAc*; ● *Man*; ● *Gal*;

Figure 2. MALDI-TOF/TOF tandem mass spectra of biantennary fucosylated *N*-glycans with bisecting GlcNAc residue recorded from lung cancer cells for the precursor ions with *m/z*: **A**) 2102.8; and **B**) 2394.9. Fragmentations schemes indicate dominant isomeric structures

Figure 3. MALDI-TOF/TOF tandem mass spectra recorded for fucosylated more antennary *N*-glycans for precursor ions with m/z: A) 2922.1 (from lung cancer cells); and B) m/z 3579.3 detected in biopsy of a patient with lung metastases. Dominant structure for each glycan is suggested based on characteristic major losses of monosaccharides residues.

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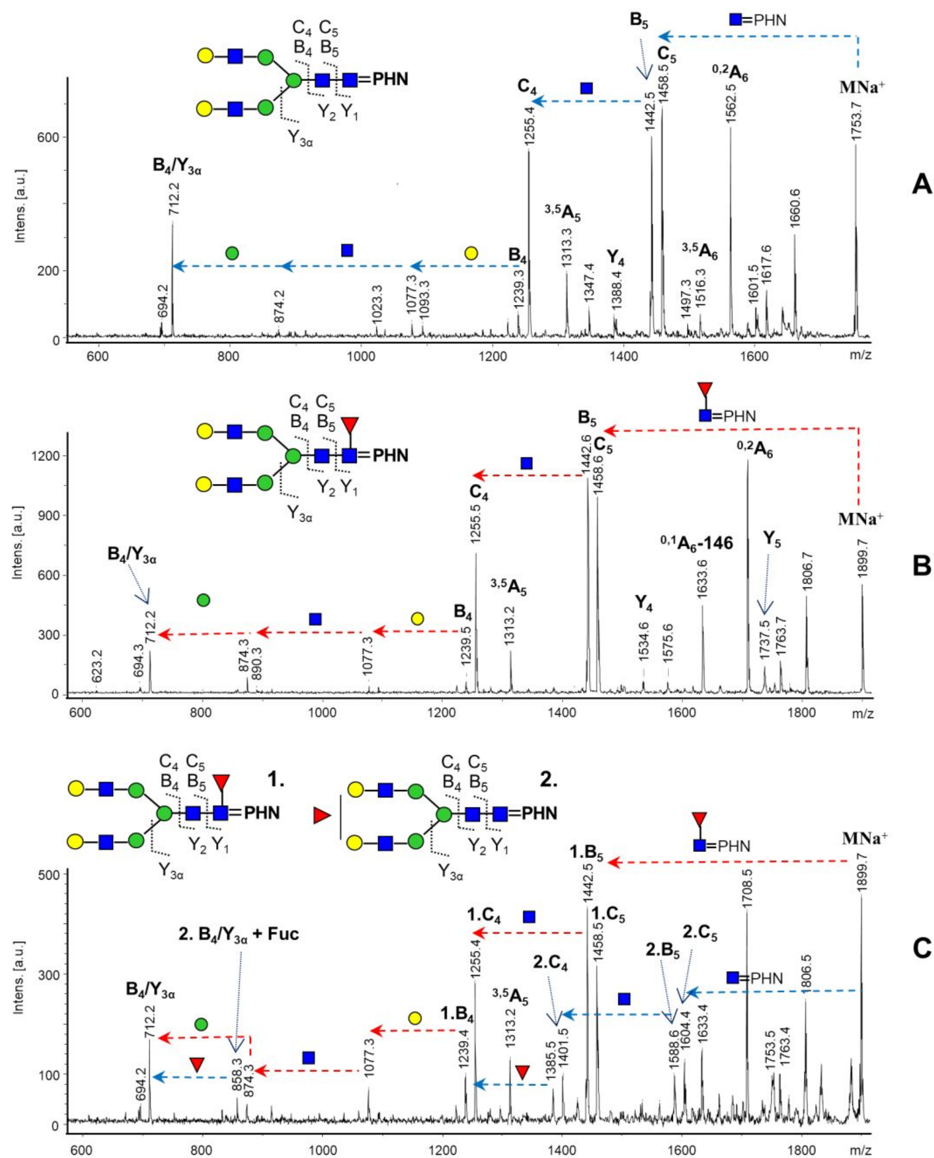

Figure 1

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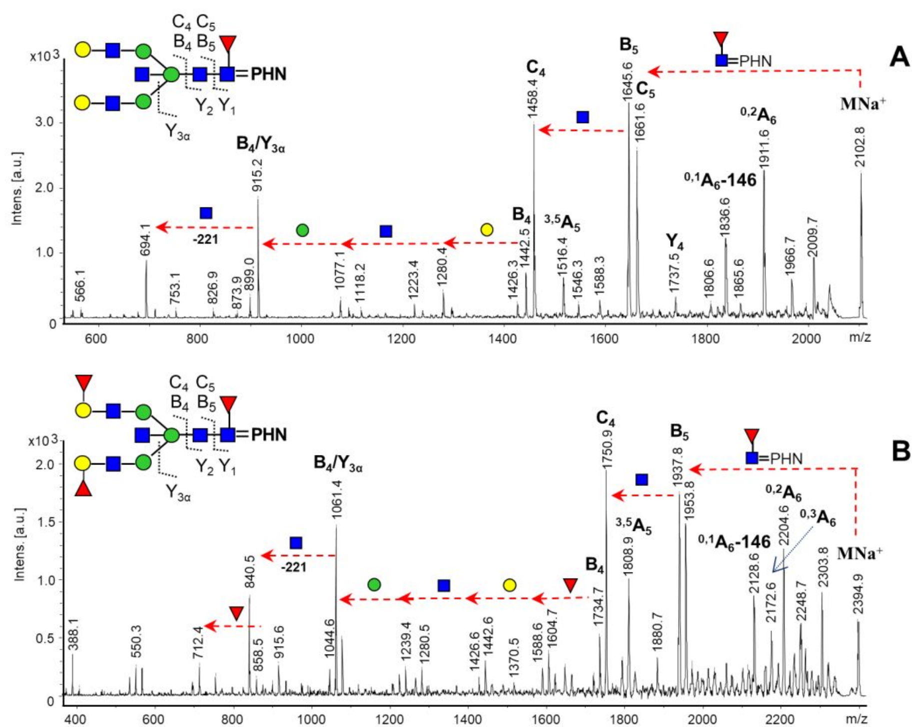

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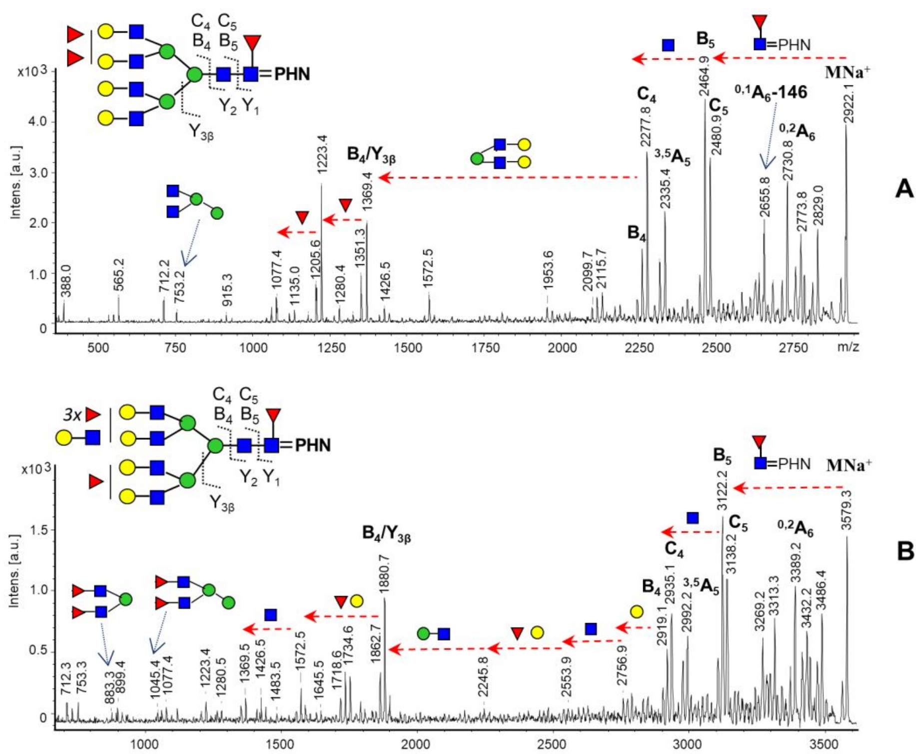

Figure 3