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Field-asymmetric ion mobility spectrometry to improve the separation and identification of released N-glycans

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ABSTRACT

Purpose. Demonstrate the application of field-asymmetric ion mobility spectrometry for selective analysis of released N-glycans.

Methods. N-glycans released from bovine fetuin and human immunoglobulin G (IgG) were analyzed in negative polarity nanoelectrospray (nESI-) using both direct infusion and HILIC liquid chromatography (LC). Analyses were performed on a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer equipped with a Thermo Scientific™ FAIMS Pro Duo interface and coupled to a Thermo Scientific™ UltiMate™ 3000 RSLCnano system.

Results Our data show that N-glycans populations are transmitted over a wide range of compensation voltages (CVs) spanning from +15 V to +65 V. Low charge state species (-1) are transmitted at low CVs below +20 V while higher charge states (-3 and -4) are the most abundant for CVs above +40 V. The nature of the transmitted N-glycans is also dependent on the CVs: for a same charge state, the transmission profiles typically go from high-mannose type content to biantennary type, and to tri/tetra-antennary type content as the CV is progressively raised. Working within an adequate CV range thus makes it possible to filter out irrelevant monocharged ions as well as background ions, resulting in a subsequent increase of the signal-to-noise ratio for peaks of interest.

INTRODUCTION

Carbohydrates are among the most abundant and diverse compounds found in nature. They are involved in many biological processes and serve as markers for various diseases. As glycosylation is understood as a non-template driven process, the resulting glycans span a wide diversity of structures with inherent variations in composition, conformation, size, and polarity. Added to low and uneven ionization efficiencies, these aspects make their analysis particularly challenging.

The FAIMS Pro Duo interface operates as a tunable ion filter offering an additional dimension of separation based on differential ion mobility. Installed at the entrance of the MS instrumentation, it reduces chemical noise and matrix interferences, resulting in improved robustness and possibly better limit of detection/quantification. The field-asymmetric ion mobility spectrometry (FAIMS) separation is conditioned by a combination of charge state, shape and size of gas phase molecular ions.

We here show how the FAIMS technology interfaced in front of an orbitrap mass analyzer can be used for preferential gas phase enrichment of released N-glycans populations according to their composition and charge states.

MATERIALS AND METHODS

Sample Preparation. N-glycans were enzymatically released from fetuin (bovine) and immunoglobulin G (human serum) glycoproteins at 10 mg/mL in solution using PNGase F kit (Gibco). Samples were purified on Thermo Scientific™ HyperSep™ Hypercarb™ SPE cartridges and evaporated to dryness. For direct infusion, samples were resuspended to 10 µM in 85:15 H2O:acetonitrile + 0.1% formic acid. For HILIC liquid chromatography, samples were resuspended in 10:90 ammonium acetate 50 mM:acetonitrile.

LC-MS method. Analysis were performed on an Orbitrap Exploris™ 480 mass spectrometer equipped with a FAIMS Pro Duo interface. For direct infusion, the instrument was equipped with a Thermo Scientific™ Nanospray Flex™ Ion Source and the samples were sprayed from Medium NanoES spray capillaries at 1.1 kV. LC analysis were performed on a Thermo Scientific™ UltiMate™ 3000 RSLCnano system equipped with a SeQuant™ ZIC-HILIC Nano LC column (150 mm x 75 µm, 5 µm, 200 Å) and a Thermo Scientific™ EASY-Spray™ Nano emitter. Detailed LC and MS settings are provided in Figure 1 and Table 1.

Data analysis. Data were processed using Thermo Scientific™ Freestyle™ 1.8 SP2 and SimGlycan™ 5.95 software (PREMIER Biosoft).

Figure 1. LC gradient

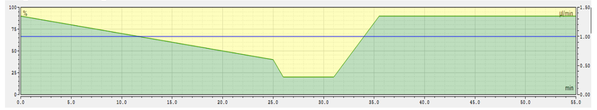


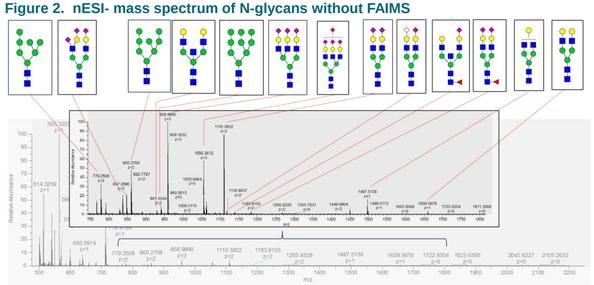
Table 1. Detailed MS and LC settings.

EASY-Spray™ Nano Ion Source		Orbitrap Exploris™ 480	
Spray voltage	- 1.5 kV	MS1/MS2 resolution	60,000 / 30,000
Ion transfer tube	150 °C	Mass range	400 - 2000 Da
FAIMS carrier gas flow	3.8 L/min	Cycle time	1.5 s
FAIMS resolution	Standard	RF Lens	80 %
		Normalized AGC target	300 % (MS1) 200% (MS2)
		Maximum injection time	150 ms (MS1) Auto (MS2)
		HCD collision E	30 %

RESULTS

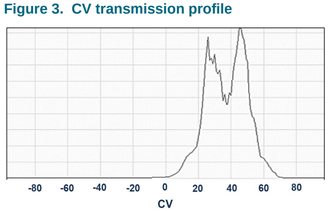
Inventory of N-glycans without FAIMS. To assess the generated populations, the diverse mixture of N-glycans released from bovine fetuin and human IgG was infused in nESI-without FAIMS and data were collected in DDA mode. RAW MS1/MS2 data were analyzed with SimGlycan™ 5.95 software to identify sampled populations. The averaged MS1 spectrum together with most abundant identified species are shown in Figure 2. These consist in a mixture of high-mannose and bi-antennary N-glycans from IgG, and complex sialylated N-glycans from fetuin.

Figure 2. nESI- mass spectrum of N-glycans without FAIMS



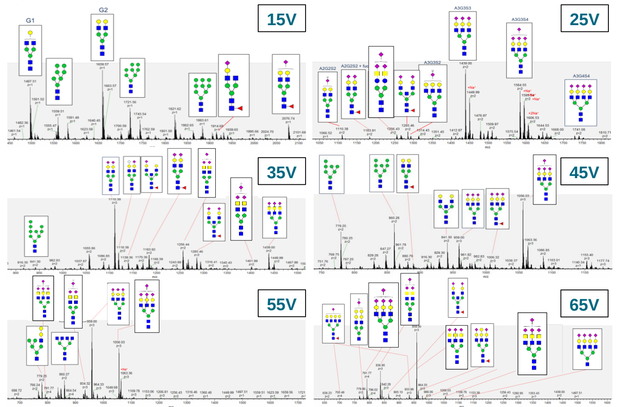
CV optimization. To evaluate the transmission profile of N-glycans with FAIMS, MS1 data were collected using the *CV scan feature* in full scan mode for CVs ranging from -100 V to +100 V by 5 V increment. A transmission profile is established by reporting the intensity of transmitted molecular ions as a function of the CV, as shown in Figure 3. Most N-glycans are transmitted between +10 V and +70 V.

Figure 3. CV transmission profile



Detailing transmitted populations per CV. The evolution in the nature of N-glycan populations transmitted according to the CV was evaluated by recording data in DDA mode at specific CVs : +15 V, +25 V, + 35 V, +45 V, +55 V and +65 V. MS1 spectra and most abundant identified species are illustrated in Figure 4.

Figure 4. Transmitted population for 6 distinct CVs



CV = +15 V mostly transmits low-mass monocharged ions consisting of high-mannose and bi-antennary N-glycans.

CVs = +25 V, +35 V and +45 V transmit different populations of doubly charged species. Within this interval, the transmission profile is mostly conditioned by the mass and is progressively shifted to lower masses as the CV is increased.

CVs = +55 V and +65 V transmit different populations of triply charged species with a similar dependence on mass. $[M\text{-}4H]^{-4}$ molecular ions are also observed at +65 V.

The transmission profiles of the most abundant N-glycans as a function of their charge are summarized in Figure 5. The different charge states are transmitted within distinct CV intervals as previously reported for lipids[1]. This feature allows to filter the signal and enhance the detection of highly charged species prior to their fragmentation. The consequence is an improved signal-to-noise ratio and a boost in identification when using the FAIMS, as in Figure 6.

Figure 6. Number of IDs w/ and w/o FAIMS

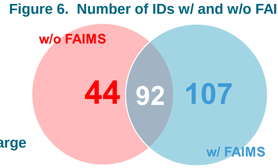
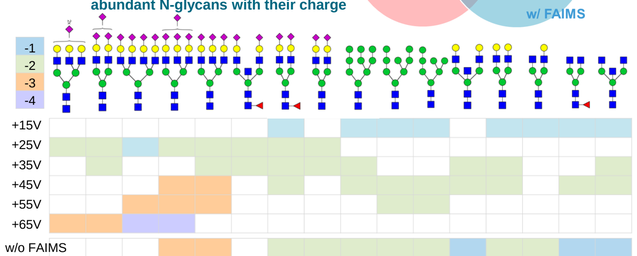
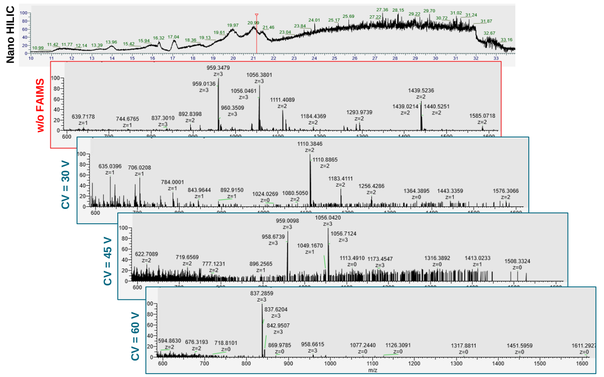


Figure 5. Transmission profile of most abundant N-glycans with their charge



Application in a LC run. The application of FAIMS for signal filtering and charge state selection was tested as part of a LC run. Our results show how FAIMS allows to selectively filter out $[M\text{-}2H]^{-2}$ and $[M\text{-}3H]^{-3}$ ions from the total population, as reported in Figure 7.

Figure 7. Sampling of a chromatographic peak w/o and w/ FAIMS at different CVs



CONCLUSIONS

Differentiation of N-glycans regarding their nature and charge state is achievable at nano flow rates using the FAIMS Pro Duo interface.

The selectivity of the FAIMS Pro Duo interface contributes to a better signal-to-noise ratio and a better resolution of isobaric species, in turn improving MS/MS spectra and leading to more confident annotation in SymGlycan™.

First results show that the FAIMS filtering is compatible with nanoflow LC run. Transfer to analytical LC flow rates could open the scope to stand-of-the-art lipidomic analyses.[2]

REFERENCES

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2. Wenjing Peng. *et al.* J. Am. Soc. Mass Spectrometry Reviews, 2023 42(2), 577-616

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TRADEMARKS/LICENSING

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