Laser-induced post-ionization for the enhanced MALDI-2-MS analysis of *N*-glycans

Bram Heijs[1,2]; Alexander Potthoff[1]; Hans Dalebout[2]; Jens Soltwisch[1,3]; Klaus Dreisewerd[1,3]

[1] Institute for Hygiene, University of Münster, Münster, Germany. [2] Center for Proteomics & Metabolomics, Leiden University Medical Center, Leiden, The Netherlands.
[3] Interdisciplinary Center for Clinical Research (IZKF), University of Münster, Münster, Germany.

b.p.a.m.heijs@lumc.nl

WWU MÜNSTER

AIM: Assess the feasibility and optimize conditions for the analysis of *N*-glycans by MALDI-2 mass spectrometry imaging

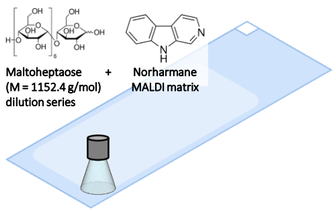
Introduction

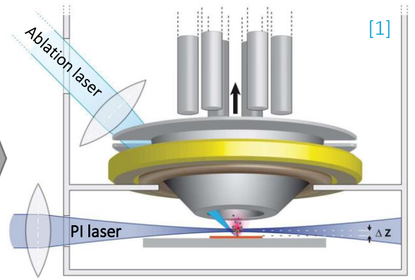
The analysis of *N*-linked glycosylation has garnered significant interest in the biomedical and clinical research communities. *N*-glycans, a class of common post-translational protein modifications, are involved in numerous cellular processes, including cell-cell interactions, and signaling. Aberrant glycosylation patterns have been associated with disease, including auto-immune diseases, and multiple cancer types. One of the most recent analysis platforms for *N*-glycans is matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI). Laser-induced post-ionization coupled to MALDI-MS, MALDI-2-MS, has recently been shown to drastically enhance ion yields and measurement sensitivity for the MALDI-MS-based analysis of (glyco-)lipids [1]. Here we aim to investigate the possible benefits of MALDI-2-MS for the analysis of polysaccharides, to ultimately improve the MALDI-MSI analysis of *N*-glycans.

[1] Soltwisch *et al.* (2015) *Science*

Experimentals

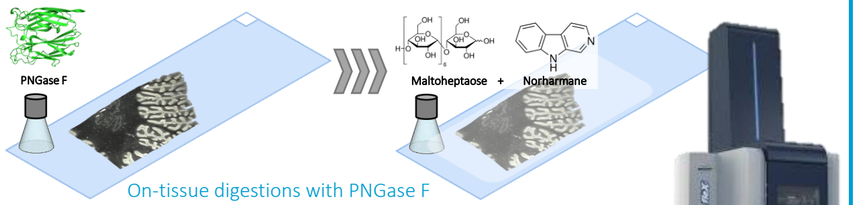
MALDI-2 parameter optimization





Optimization of laser pulse energies, inter-laser delay, and cooling gas pressure

N-glycan MALDI-2-MSI



On-tissue digestions with PNGase F
Overnight at 37 °C in a saturated humid environment

Bruker Daltonics MALDI-2 timsTOF flex

QTOF-MS with trapped ion mobility separation module, modified for MALDI-2
Ablation laser: 10 kHz Bruker smartbeam 3D (355 nm)
PI laser: 1 kHz Ekspla N204-1k-FH (266 nm, 7 ns pulse length)
Pressure: 200 - 350 Pa

Results & Discussion

MALDI-2 parameter optimization on maltoheptaose standards

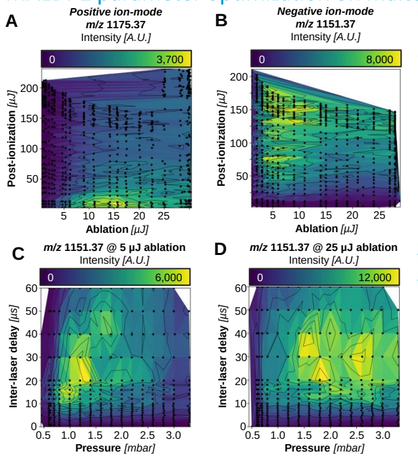


Figure 1: **(A)** In positive ion-mode sodium adducts did not benefit from MALDI-2. **(B)** In negative ion-mode deprotonated ions showed a beneficial intensity boost by MALDI-2. Optimization of the inter-laser delay (ILD) at low **(C)** and high **(D)** ablation laser intensity (ALI) revealed the presence of two ion populations. One fast moving population of single ions formed through thermal desorption (low ALI: < 100 Pa, ILD 10-20 µs) and one slower moving population of ablation clusters (low ALI: > 1 mbar, ILD 20-40 µs; high ALI: > 150 Pa, ILD 20-40 µs). Post-ionizing the ablation clusters resulted in the highest ion yields.

MALDI-2 measurement sensitivity assessment

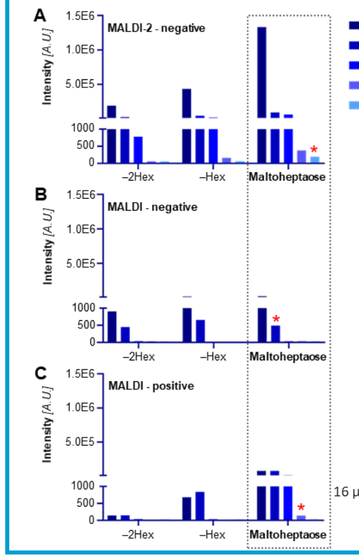


Figure 2: A dilution series of maltoheptaose was sprayed on glass slides and analyzed by **(A)** MALDI-2-MS in negative ion-mode, **(B)** MALDI-MS in negative ion-mode and **(C)** MALDI-MS positive ion-mode.
The lower limit of detection (LLOD: S/N ≥ 3) for MALDI-2 (A) was three orders of magnitude lower in MALDI-2 compared to MALDI in negative ion-mode (B), and one order of magnitude compared to the current positive ion-mode gold standard (C).

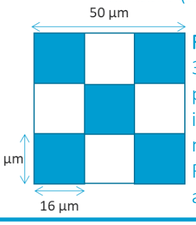


Figure 3: The smartbeam 3D "M5 small" ablation pattern. The ablation area is 1280 µm². Amounts of maltoheptaose per pixel in Figure 2 are based on this ablation pattern.

Negative ion-mode MALDI-2-MSI and *in-situ* MALDI-2-MS/MS of *N*-glycans in formalin-fixed, paraffin-embedded human brain (cerebellum)

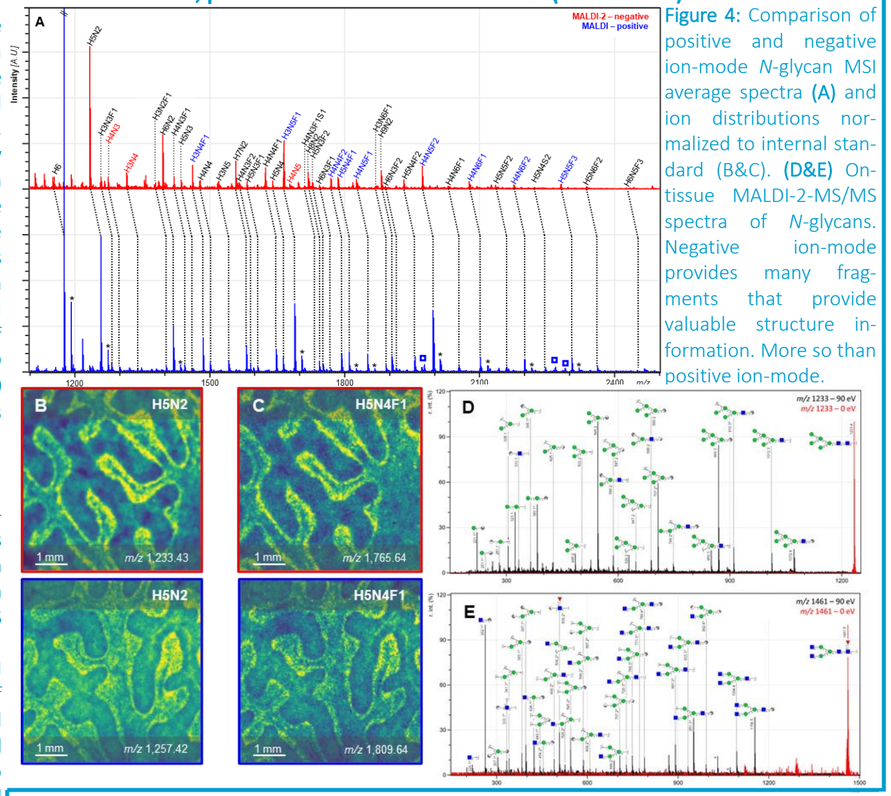


Figure 4: Comparison of positive and negative ion-mode *N*-glycan MSI average spectra **(A)** and ion distributions normalized to internal standard (B&C). **(D&E)** On-tissue MALDI-2-MS/MS spectra of *N*-glycans. Negative ion-mode provides many fragments that provide valuable structure information. More so than positive ion-mode.

Conclusions

- MALDI-2-MS of deprotonated oligosaccharides provides substantial boosts in ion yields
- Optimal MALDI-2 conditions are:
  - Cooling gas pressure: 250 – 300 Pa
  - Inter-laser delay: 30 µs
- MALDI-2 provides high quality MALDI-MSI and on-tissue MS/MS data for *N*-glycans directly from tissue

Acknowledgements

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