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

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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 autoimmune 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.


Experiments
MALDI-2 parameter optimization

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

N-glycan MALDI-2-MSI

On-tissue digestion with PNGase F

Overnight at 37 °C in a saturated humid environment

Brucker Daltonics MALDI-2 timsTOF flex

QTOF-MS with trapped ion mobility separation module, modified for MALDI-2
Ablation laser: 10 kHz Brucker smartbeam 3D (355 nm)
Pi laser: 1 kHz Ekspla N2O4-1k-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 maltose was sprayed on glass slides and analyzed by (A) MALDI-2-MS in negative ion-mode, (B) MALDI-2-MS in positive ion-mode and (C) MALDI-2-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).

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
The authors would like to thank Brucker Daltonics for funding and assistance during the project.