(Nano)-LC coupled to Ion Mobility Q-TOF for an improved sensitivity and proteome coverage

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INTRODUCTION

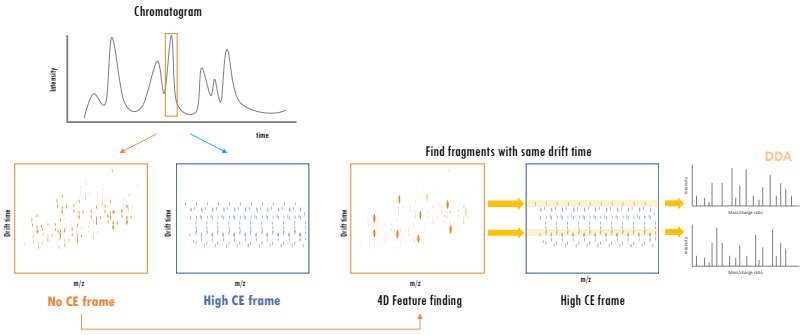
Ion mobility mass spectrometry (IMS) is a state-of-the-art technique that first separates gas phase ions based on their mobilities and then based on their m/z ratio. IMS is known to refine omics workflows by providing cleaner MS and MS/MS spectra thereby facilitating the analyte identification, reducing the false discovery rate and at the end improving the confidence in results. In addition, IMS improves the data independent (DIA) workflow by applying drift time-based collision energy thereby drastically improving the spectra quality of the detected features in proteomics studies compared to classical all ions methods where a fixed collision energy is applied.

In this study we compared the ability of two acquisition modes (classical DDA mode vs. DIA mode) available on the IMS-Q-TOF instrument and two ionization sources (nano-ESI source vs. ESI) to resolve increasing quantities of complex proteomics samples (*E. Coli* digest). The identified peptides were compared and the complementarity between acquisition modes and sources were highlighted.

OPERATIONAL CONDITIONS

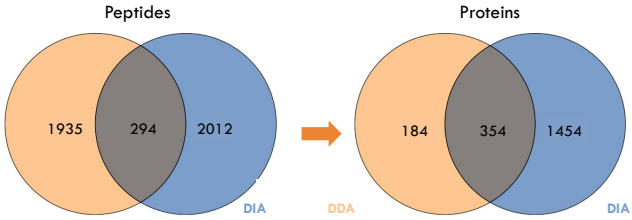
IM-MS acquisition mode

- Ion mobility Q-TOF (Agilent Technologies)
- DIA with alternating frame mode
- Collision energy applied based on drift time
- Computationally aligned precursors and fragments
- Peptide sequencing using Spectrum Mill



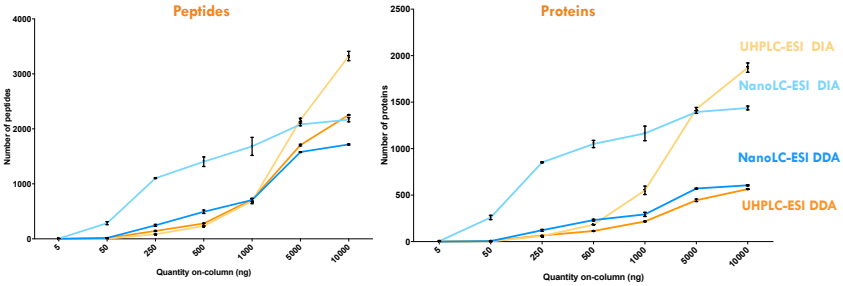
COMPLEMENTARITY OF ACQUISITION MODES

- UHPLC-ESI
- 10 µg of *E.Coli* digest on-column
- Peptides and proteins detected in at least 2 out of 3 replicates



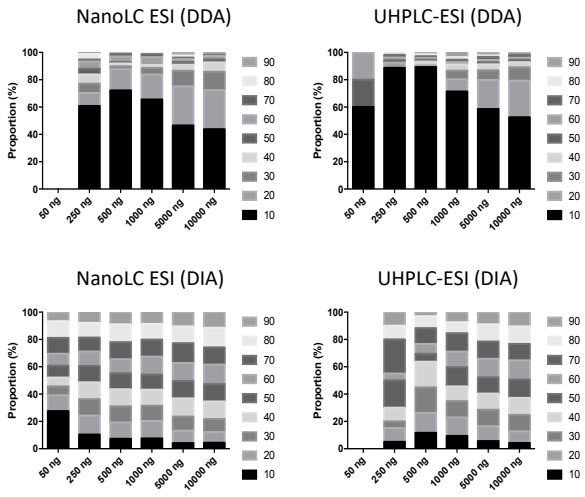
DETECTED ENTITIES

- Detected peptides and proteins using both separation techniques and acquisition modes
- Each condition was realized in triplicate



PROTEOME COVERAGE

- Endogenous proteins classified by their natural abundances in *E. Coli*
- Classification in deciles
- 10% most abundant proteins account for 88.4% of total protein content
- Unique proteins detected in at least 2 out of 3 replicates



CONCLUSIONS

- Comparison of 2 acquisition modes and separation techniques
- Nano ESI is more sensitive than ESI and therefore more suitable for protein contents below 1 µg
- UHPLC-ESI is more interesting to use for protein quantities above 1 µg on column
- DIA offers the possibility to detect more proteins compared to DDA
- DDA is clearly biased towards highly abundant proteins
- Thanks to the absence of precursor selection, DIA allows to detect proteins coming from a wider concentration range than DDA
- Both acquisition modes are complementary and provide additional information to address the biological challenge

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