Pre-Sliced diaPASEF: Narrowband accumulation in the first timsCell for Ultra-Sensitive Plasma Proteomics

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Introduction

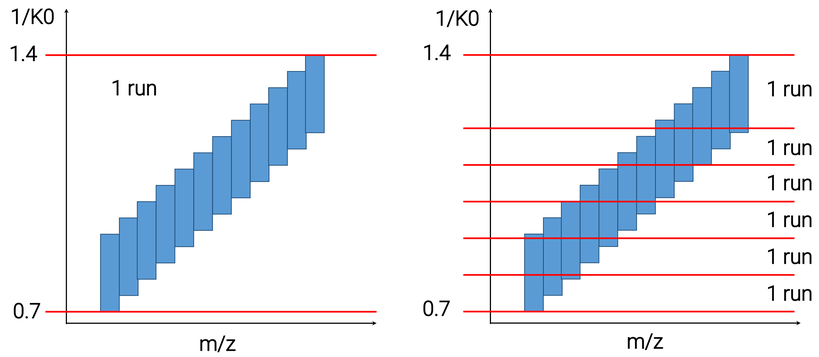
Mass spectrometry-based proteomics is a powerful methodology that is nowadays able to detect and quantify thousands of proteins in cells, tissues and biologic fluids. However, the accurate quantification of very low abundant proteins, in biological fluids where the dynamic range spans over 10 orders of magnitude, remains a challenge. The timsTOF Pro family of mass spectrometers, with the introduction of the Trapped Ion Mobility Separation (tims) dual cell, has greatly improved sensitivity by separating ions by ion mobility, especially when a high number of co-eluting compounds are simultaneously targeted. In an attempt to further enhance sensitivity, we present pre-sliced dia-PASEF, a new methodology by narrowing the ion mobility range of ions injected in the second tims cell, hence greatly increasing sensitivity.

Methods

A pool of synthetic peptides of low abundant proteins was spiked in digested non-depleted plasma samples ranging from 10 fmol to 0.64 amol in a 1:5 dilution series while the heavy synthetic peptides were spiked in all samples at 10 fmol. Samples were then analyzed using a timsTOF Pro (Bruker) coupled to a nanoElute2 nano-HPLC (Bruker). Samples were separated using 60 min, 40 min, 20 min and 10 min gradients, either with an ion mobility window from 0.7 to 1.4 1/k0 (wide range) or using narrower 0,1 1/k0 width windows, ranging from 0.7 to 1.25 in a g-DIA-PASEF mode. Data was analyzed using Skyline.

Results

The pre-sliced dia-PASEF methods has been created by dividing the ion mobility range of the standard g-dia-PASEF method into 0,1 V/cm^2 slices, each slice being handled as an independent (for the initial test purposes) dia-PASEF acquisition (Figure 1). In these methods, the first tims cell parameters. are set in a way that prevents low 1/K0 ions to enter the first tims cell, hence acting as a high pass filter. This consequently reduces the number of charges reaches the second tims cell, and the purpose was to check whether this selective enrichment could yield higher sensitivities. In non-depleted plasma, the dynamic range spans over 10 orders of magnitude, with a handful of proteins making up 99% of the sample. Quantifying very low abundant proteins like cytokines in non-depleted plasma samples is thus a challenge (Figure 2). Using 60 min gradients we observed that peptides that can't be identified or quantified in the wide ion mobility range are identified and quantified in the narrower windows corresponding to their mobility, in some cases with up to 4x increase in sensitivity, at the amol level. In fact, we could observe the increasing intensity of peptides throughout the narrower windows with peptide intensity reaching its maximum in the specific windows of the peptide mobilities (Figure 3), while quantitation quality is preserved (Figure 4)..



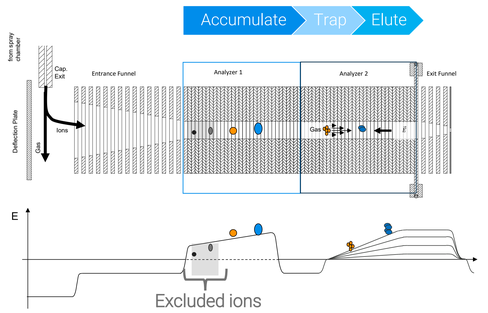


Figure 1: Pre-slicing the initial g-dia-PASEF method into 6 distinct runs with narrow ion mobility window
Working on narrower ion mobility allows on the one hand to remove the higher mobility ions from the pool of trapped species and on the other hand to increase the ion mobility resolution by spending more elution time on a narrower span of ion mobilities.
Right hand : higher ion mobility ions are excluded by raising the first tims cell inlet potential.

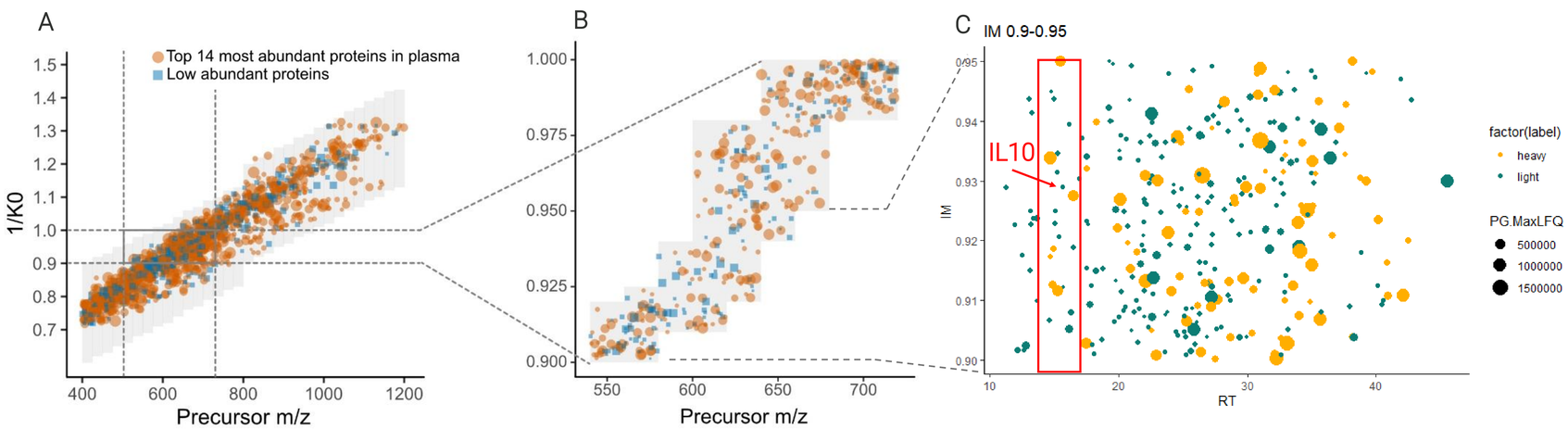
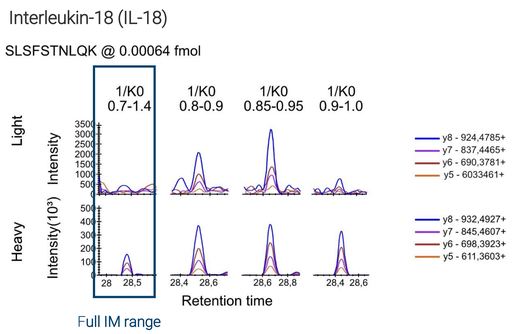


Figure 2: Spreading complexity over a narrow ion-mobility g-dia-PASEF window
A: reverse ion mobility vs m/z heatmap as a fixed 100ms time point of the gradient for a neat plasma Injection. Brown dots are representing peptides issued from the top14 most abundant proteins while blue squares are representing the targeted cytokine peptides .B: display of the new pre-sliced g-dia-PASEF windows disposition, covering the narrow 0,1 1/K0 reverse ion mobility range, C: Zoom on the 0,9-0,95 reverse ion mobility region, highlighting the decreased sample complexity with a focus on IL 10 peptide



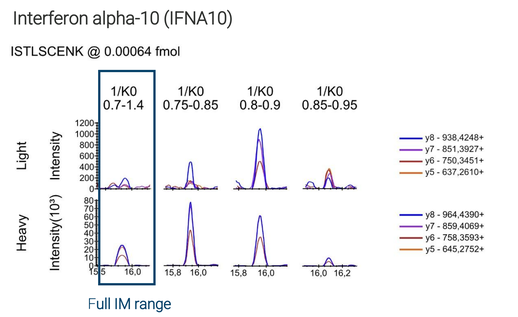


Figure 3: Sensitivity increase within pre-sliced g-dia-PASEF runs
Exemple of IL-18 and INFA10 peptides spiked in undepleted plasma @ 10fmol/µl (heavy form) and 640 zmol/µl (light form). 1µl injected.

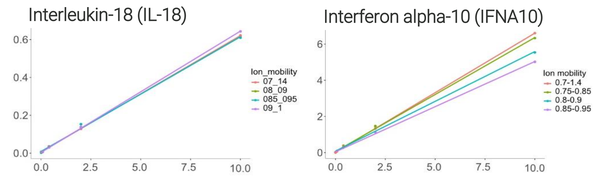


Figure 4: Quantitation : linear over >4 orders of magnitude
Exemple of IL-18 and INFA10 peptides

Conclusions

- Pre-sliced g-dia-PASEF has proven to be an effective way to increase the sensitivity of targeted experiments
- Clinically relevant could be detected and quantified from undepleted plasma @ sub-attomolar levels on column
- Further experiments will expend the target list and refine the method

Targeted proteomics

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