Fast nanoLC separations for high throughput body fluid analysis with a TIMS equipped QTOF and 4D feature alignment

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Introduction

Body fluids provide the easiest possibility to monitor physiological human parameters in depth; they are routinely collected with minimal or no invasiveness and can also be obtained through biobanks from thousands of clinical samples. To maximize throughput, we have optimized MS conditions, column lengths and LC overhead times to obtain runs of 28.8 min injection to injection (50 samples/day). Additionally, we utilized PEAKS X software which aligns features in four dimensions; retention time, intensity, m/z and ion mobility to transfer identifications in a match between run design for enhanced data completeness in data dependent acquisitions across runs. This investigation was performed on the Bruker nanoElute but similar results can be achieved on the Evosep One together with PASEF, as recently described[1].

Methods

Plasma proteome (PP) tryptic digests (top 12 depleted) were kindly provided by Roman Fischer (Oxford University). Human colostrum samples (HC) were collected over the first 14 days after birth and were digested with trypsin. 50 ng of tryptic digests from PP and HC were delivered to a 100 mm fritted column (Bruker TEN) that was connected to a zero dead volume emitter (Bruker, ID 20 µm fused silica emitter). A High-resolution timsTOF Pro mass spectrometer utilizing the PASEF acquisition method was used and the PASEF cycle was set to 0.5 s equating to 100 a ms TIMS MS scan followed by four 100 ms PASEF MS/MS cycles, each fragmenting on average 12 precursors. Data were analyzed using PEAKS X (Bioinformatics solution Inc.). Results were filtered to 1% FDR (PSM), and matching was applied with a window of 0.05 ($1/K_0$) and 1 min (RT). Protein profile plots were generated in Perseus v.1.6.0.6 (Cox group) and the proteomaps on a web based application (www.proteomaps.net)[2].

1)Kosinski T., Heilig R., Bensaddek D., Bache N., Hørning O., Fischer R., Koch H., Plasma proteomics goes high throughput – timsTOF Pro with PASEF and 4D feature alignment to quantify 500 plasma proteins in 11.5 min (Bruker Application Note)

2) Liebermeister W., Noor E., Flamholz A., Davidi D., Bernhardt J., and Milo R. (2014), Visual account of protein investment in cellular functions, PNAS 111 (23), 8488-8493.

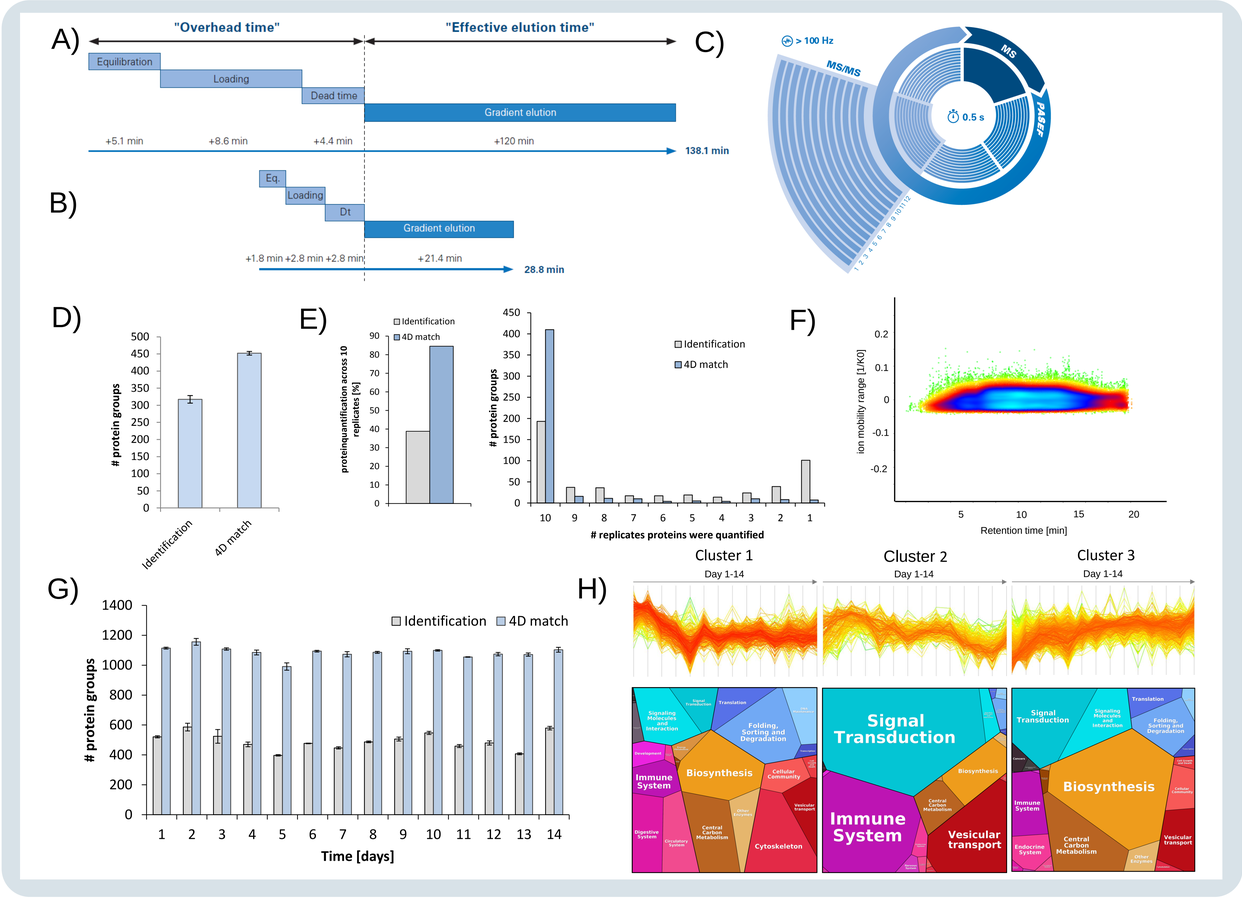

Figure 1: A) On 120 min nanoLC gradients with direct loading, overhead times take 18 min B) reduction of overhead times by optimized loading and equilibration. C) PASEF acquisition scheme, achieving a duty cycle of 0.5 s while still acquiring MS/MS at ~100 Hz. D) IDs on depleted plasma digests (average 10 runs). E) Data completeness of protein groups quantified by using a 4D match approach (>85%) on 10 runs. F) Low deviation of 1/K0 values for matching between runs. G) ID numbers for postnatal investigation of the composition of human colostrum over the first 14 days using a 4D matching approach and regular PASEF and MS/MS IDs. H) Three major protein abundance time profiles were extracted with proteins involved in the immune system, biosynthesis and signal transduction.

Conclusions

- Reduced nanoLC overhead times enable efficient use of MS instrument time unfolding the possibilities of a fast instrument with PASEF to full potential
- Transfer of IDs between runs by using 1/K0 as an additional dimension adds confidence on matched results and reduces missing values across runs
- Application on bodyfluids (plasma, colostrum) illustrates one of several use cases to run PASEF in a match between runs design in large cohorts of samples

timsTOF Pro