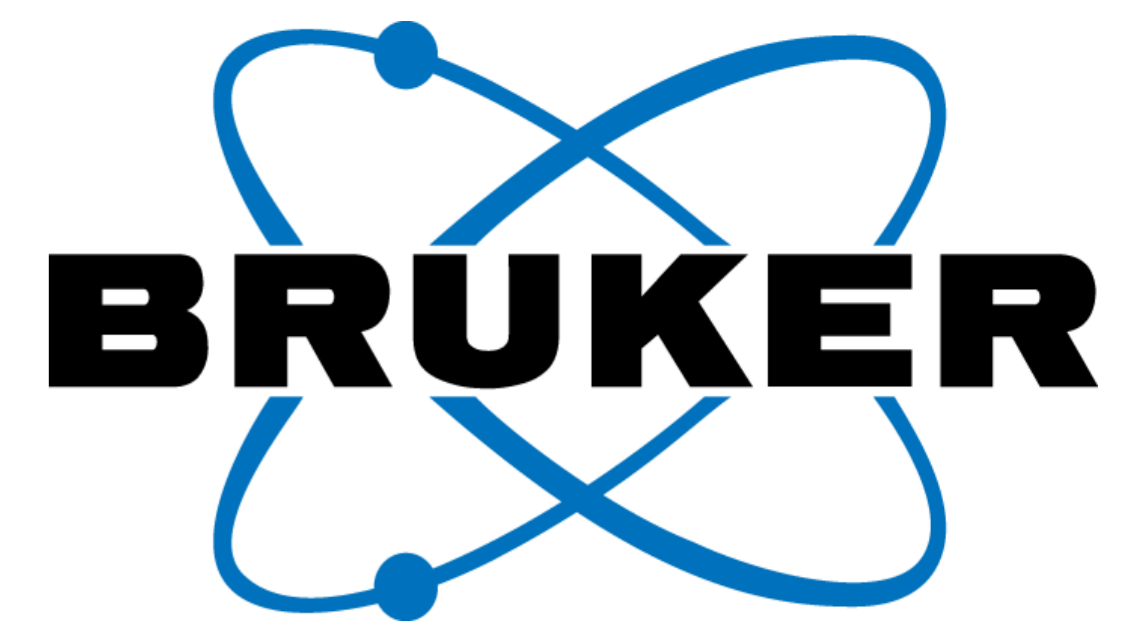# High throughput plasma proteomics with PASEF and 4D feature alignment

BRUKER

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Introduction

Body fluids provide the easiest possibility to monitor in depth physiological human parameters; they are routinely collected with minimal or no invasiveness and can also be obtained through biobanks from thousands of clinical studies. 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 to enhance data completeness across runs. This investigation was performed on the Bruker nanoElute, 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)[1]. Human colostrum samples (HC) were collected over the first 14 days and digested with trypsin in an protease : substrate ration of 1:50. 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 one 100 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 on the level of PSMs on the ID result. 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].
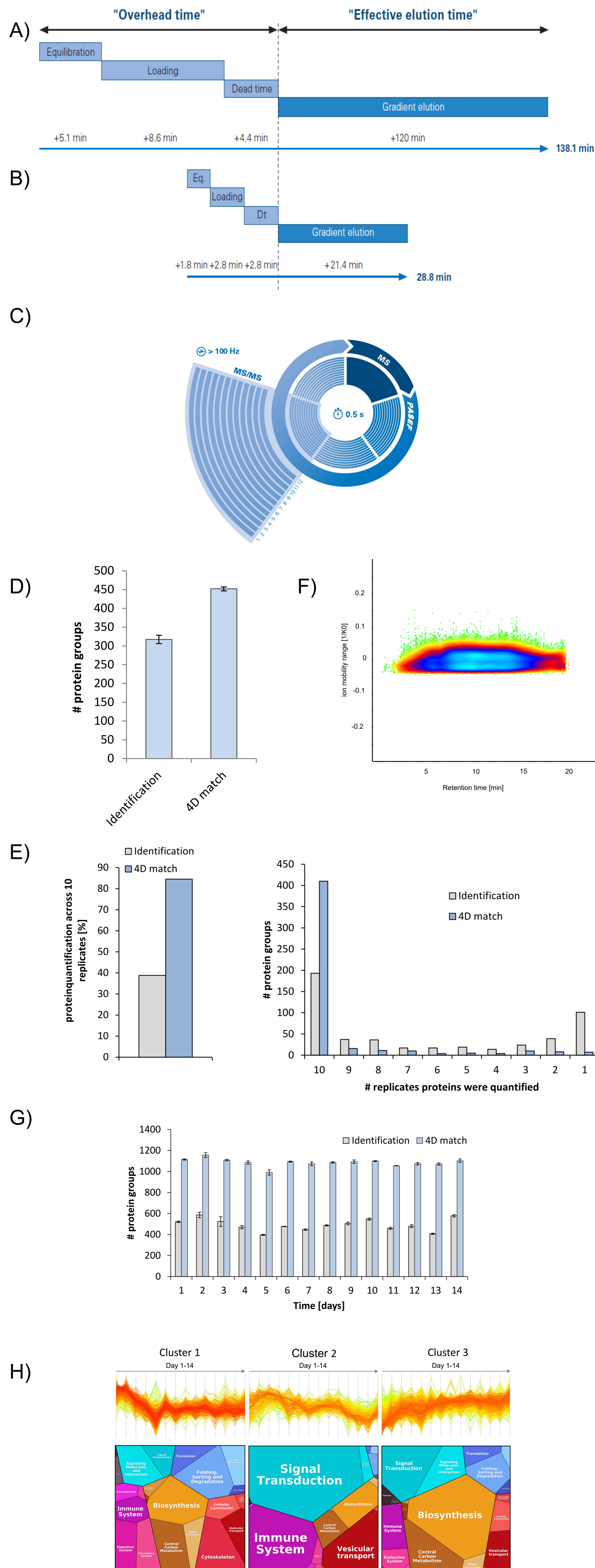

Figure 1:

A) On 120min LC 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.
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.

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.

Conclusions

- Reduced LC overhead times enable efficient use of MS instrument time of an instrument running very fast with PASEF

- Transfer of IDs between runs by using rt, m/z and 1/K0 adds additional confidence and allows boosting ID numbers.

- Application on body fluid samples (plasma, colostrum) to run at high throughput of 50 samples/day illustrated on blood plasma and human colostrum.

timsTOF Pro