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High-Throughput Ultra-Low Flow LCMS platform for low sample amount proteome profiling

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ABSTRACT

Single cell-like bottom-up proteomics experiments were conducted using diluted bulk cell lysate protein digests. By coupling a dedicated low-input reversed phase nanoLC column to a state-of-the art Orbitrap based mass spectrometer and utilizing variable flow rate solvent gradients, high throughput ultra low flow rate separations could be achieved. We demonstrate that pairing the analytical column with a dedicated trapping column allows pushing throughput even further at sub-100 nL/min flow rates, resulting in effective instrument productivity close to 70% for 100 samples per day. Removal of singly charged background ions using differential ion mobility, increases proteome coverage up to 40%, resulting in consistent identification of more than 2000 protein groups from as little as 250 pg of protein digest standard.

INTRODUCTION

Recent developments in LC-MS instrumentation have been pivotal in paving the way for the single cell revolution. Improvements at different stages in the workflow have pushed sensitivity at least one order of magnitude and have leveraged automated workflows with near loss-less sample processing. These developments are key to moving towards standardized workflows and large-scale single cell MS studies. From a separation point of view, operation of capillary bore LC columns at ultra-low flow rates yields the highest absolute sensitivity, but these workflows are inherently limited in terms of sample throughput, instrument productivity and workflow robustness. In the current contribution, we present a workflow capable of delivering robust low nanoflow separation with minimal instrument overhead and excellent coverage for low input samples.

MATERIALS AND METHODS

Sample Preparation

Thermo Scientific™ Pierce™ HeLa Protein Digest Standard was dissolved in 1% Acetonitrile, 1% DMSO with 0.1% TFA to a concentration of 25 ng/µL for direct injection experiments and to 1 ng/µL for trap and elute experiments.

Figure 1. Experimental setup

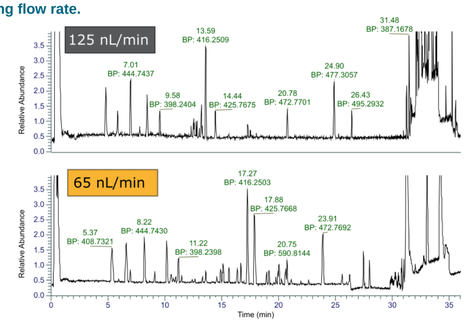
LC-MS settings and data processing

Active solvent gradients of 8, 12 and 32 min were evaluated at flow rates of 125 and 65 nL/min. Injection volumes were varied between 10 and 250 nL. Electrospray ionization voltage was set at 1,9 kV. FAIMS was operated with a single compensation voltage at -50V and a total carrier gas flow rate of 0.9 L/min. MS data were collected in data-dependent acquisition mode (Top10) with full scan data collected at 120,000 resolution and fragmentation data collected at 60,000 resolution. Quadrupole isolation width for MS2 acquisition was set at 5 Th. Maximum injection time (MaxIT) was set at 118 ms. The scan range used was 375-1500 m/z. Dynamic exclusion was set to 10 ppm with a 20 second duration. Fragmentation was performed using HCD with a fixed collision energy of 30. The acquired raw data files were processed with Thermo Scientific™ Proteome Discoverer™ software with the CHIMERYS intelligent search algorithm.

RESULTS

Increased ionization efficiency at ultra low flow rates

Figure 2. Comparison of the basepeak traces obtained for the separation of 250 pg HeLa digest using 32 min gradient methods with respectively 125 and 65 nL/min as eluting flow rate.



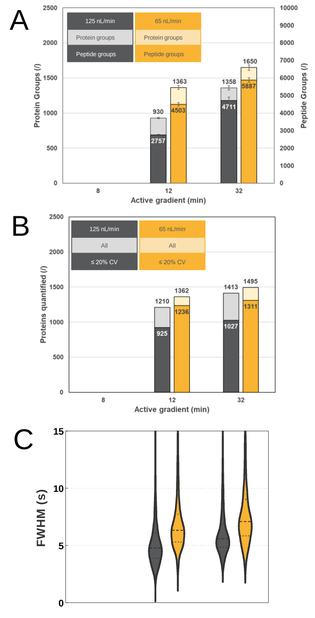
High throughput and maximum sensitivity can be combined by using variable flow rate methods with high flow rate (750 nL/min) applied during the first 2 min of the gradient, and low flow rate (125 or 65 nL/min) applied as soon as analytes start eluting.

Ionization efficiency is significantly increased when reducing the eluting flow rate down to 125 and even further down to 65 nL/min (Figure 2). For low input proteomics experiments where precursor ion concentrations tend to fall below the limit of detection, this can have a great impact as more ions can be collected for fragmentation and identification.

By reducing the eluting flow rate from 125 to 65 nL/min, we were able to increase proteome coverage by 47 and 22% for respectively 12 and 32 min gradient analyses. For bulk HeLa cell digest amounts considered to be equivalent to the amount present in a single cell (250 pg), up to 1650 protein groups could be identified on a standard nanoflow LC-MS setup (no FAIMS) with 80% instrument productivity (Figure 3).

As reducing the eluting flow rate inherently results in some loss of chromatographic performance (Figure 3C), increases in proteome coverage are predominantly attributed to the increase in ionization efficiency. Moreover, subtle improvements in quantitation (90% vs 75% below CV 20) could potentially also be attributed to this observation (increase in datapoints per peak).

Figure 3. DIRECT INJECTION results. (A) Protein and peptide group ID's. (B) Quantified proteins. (C) FWHM distribution of eluting peptides



Trap and Elute increases sample throughput and instrument productivity

Figure 4. Direct Injection versus (A) Trap and Elute (B) configuration on Vanquish Neo. 12 min active gradient profile for 65 nL/min elution, (C) Direct Injection, (D) Trap and Elute. (E) Instrument productivity versus active gradient for both configurations.

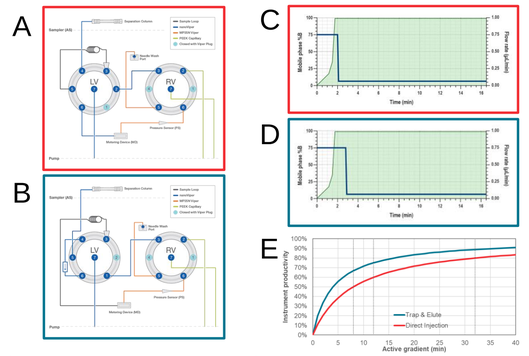
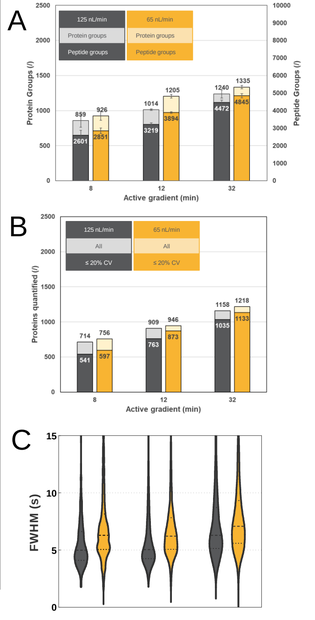


Figure 5. . TRAP AND ELUTE results. (A) Protein and peptide group ID's. (B) quantified proteins. (C) FWHM distribution of eluting peptides



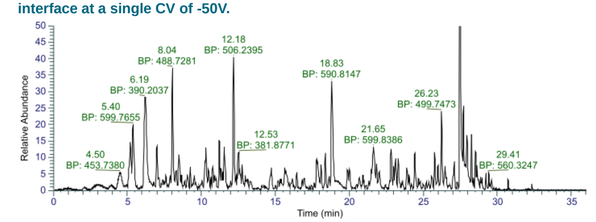
Even though the Vanquish Neo system uses optimized injection and column equilibration procedures (both at high flow rate or maximum allowed column pressure), instrument overhead can have a significant effect on the total instrument productivity (Figure 4E). When using optimized direct injection methods on the 50 cm µPAC Neo low load column (column volume 1.5 µL), minimum overhead time is 8 min. By changing the fluidic configuration to trap and elute and pairing the analytical column with a matching trap column (µPAC Neo low-load trapping column), column equilibration can be performed in parallel to sample loading, reducing the overhead time by a factor of 2 (to 4 min). Instrument productivity can be increased from 50 to 70%, from 60 to 75% and from 80 to 90% for methods with respectively 8, 12 and 32 min of active gradient.

Compared to direct injection methods, slightly lower proteome coverage was obtained (10-20%, Figure 5A), but higher throughput could be achieved. The decrease in coverage can be attributed to loss of hydrophilic peptides during sample loading, as only minor effects on peak with are observed (max increase is 10%) .

In line with the direct injection methods, the analyses at 65 nL/min consistently gave higher absolute ID numbers (8-9% on the protein group level) as those performed at 125 nL/min. Finally resulting in 926 protein groups identified and 597 proteins quantified (CV ≤ 20%) using an active gradient of 8 min at a throughput rate of 100 runs per day.

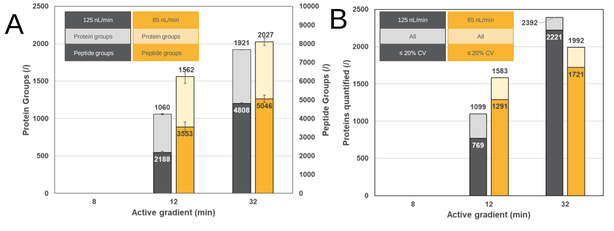
FAIMS removes singly charged background ions and increases proteome coverage

Figure 6. Basepeak chromatogram obtained for the separation of 250 pg HeLa digest using 32 min gradient methods with 125 nL/min as eluting flow rate and FAIMS pro interface at a single CV of -50V.



A significant gain in sensitivity can be achieved by using FAIMS to favour the transmission of ions with multiple charges into the MS. This is demonstrated in Figure 6 where a basepeak chromatogram obtained for the separation of 250 pg HeLa digest at 125 nL/min is shown. Even though the absolute intensity is slightly lower as compared to analyses without FAIMS, the elution window is packed with features that would otherwise have been masked by singly charged background ion signals. Up to 40% more protein groups were identified when comparing the direct injection low flow analyses, resulting in consistent identification of 2027 protein groups (technical triplates) and reliable quantification (CV≤20%) of over 2000 proteins.

Figure 7. DIRECT INJECTION RESULTS with FAIMS pro interface. (A) Protein and peptide group ID's. (B) Quantified proteins.



CONCLUSIONS

- Low nano flow rates (125/65 nL/min) can be used with µPAC Neo columns to boost sensitivity for low input samples.
- Optimized flow rate programming allows obtaining 80% instrument productivity with 32 min of active sub100 nL/min flow rate elution.
- Sample throughput and instrument productivity can be increased by using dedicated low-load trapping column.
- Below 15 min active gradients, trap and elute has the highest impact on instrument productivity.
- FAIMS can successfully be implemented at these low flow rates and increases proteome coverage even further.

TRADEMARKS/LICENSING

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