Robust Microflow LC-MS/MS Applied to Bottom-up, Middle-down, and Top-down Proteomics.

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Overview

Goal: Comparison of performance evaluation between FlowChips (micro-flow rate) and in-house packed columns (nano-flow rate) for the separation of peptides and intact proteins.

Methods: PicoSure, BSA, and digested Hela cell lysate were used for bottom-up proteomics. Digested and reduced SiLu antibody for middle-down proteomics. Top-down standards, Hela cell lysate, and intact histones for top-down proteomics. Samples were analyzed on a Dionex Ultimate 3000 using an in-house packed (PLRP-S or C-18) column system (75 µm x 10 cm) or FlowChip columns (C18 or C4, 150 µm x 10 cm) at a flow rate of 0.3 and 1.5 µL/min, respectively. The LC system was coupled to an Orbitrap Eclipse mass spectrometer. MS parameters were adjusted according to the sample being analyzed, and each sample was analyzed in triplicate. Performance evaluation included the monitoring of retention time and selectivity. Peptide, and protein/proteoform identification were searched using Mascot and TDPortal respectively.

Key points:

- **Standardization of retention time:** accurate quantification of select proteins in complex samples from LC-MS runs is critical for advances in biomarker`s discovery.
- **Nano-flow liquid chromatography (nLC)** is the method of choice for MS-based proteomics. Low flow rates improve ionization and sensitivity but becomes challenging due to its propensity to clogging and lack of reproducibility.
- **Micro-flow liquid chromatography (mLC)** is fundamentally more straightforward to work and more robust than nLC, showing excellent reproducibility.
- We demonstrate the application of mLC for the analysis of peptides, intact proteins, and antibodies using the state of art of LC-MS/MS.

Bottom-up MS

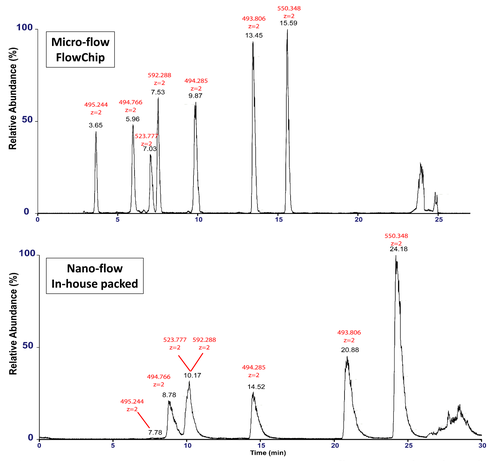

Figure 1. PicoSure separation using micro-flow rate (top) and nano-flow rate (bottom).

- Peptides from BSA digests were separated in a 60 min.-gradient run. Protein coverage between micro and nanoflow rates were comparable and higher than 75%.
- Peptides from Hela digests were separated in a 90 min.-gradient run and indicates an increase in the detection of unique peptides for mLC.

Table 1. Comparison between peptide and protein coverage at different flow rates.

	BSA		Hela	
	Peptide IDs	Protein Coverage (%)	Peptide IDs	Protein IDs
Micro-flow FlowChip 1.5 µL/min	333	86.3	9,412	1,507
Nano-flow 0.3 µL/min	213	80.7	7,687	1,715

Table 2. Comparison between protein and proteoform coverage at different flow rates.

	Histones	
	Protein IDs	Proteoform IDs
Micro-flow FlowChip 1.5 µL/min	49	464
Nano-flow 0.3 µL/min	60	416

- Intact histones were separated in 60 min.-gradient run.
- Micro and nano-flow rates show comparable results.

Middle-down MS

- Digested and reduced SiLu antibody were separated using a **15 min-gradient.**
- Similar results could be obtained nano-flow rates, but not achieving the same selectivity.

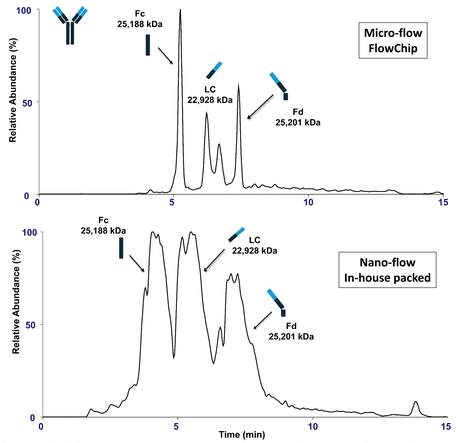

Figure 2. Digested and reduced SiLu antibody using micro-flow (top) and nano-flow rate (bottom).

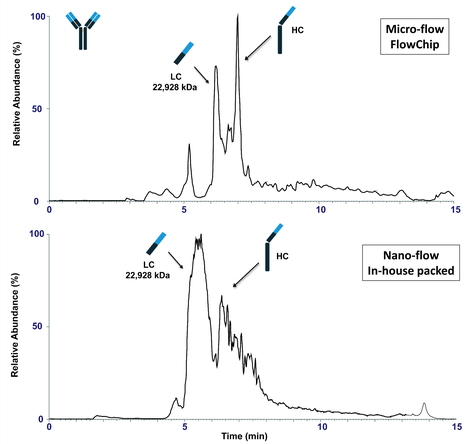

Figure 3. Reduced SiLu antibody using micro-flow (top) and nano-flow rate (bottom).

Top-down MS

- Ubiquitin, Trypsinogen, Myoglobin and Carbonic Anhydrase were separated using a **15 min-gradient.**
- Similar results could be obtained nano-flow rates, but not achieving the same selectivity.

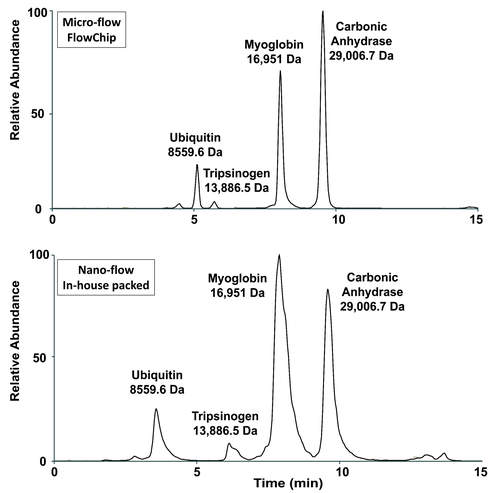

Figure 4. Separation of top-down standards using micro-flow (top) and nano-flow rate (bottom).

- Hela lysates were fractionated to cutoff proteins bigger than 30 kDa.
- Proteoforms from Hela lysates were separated in a 60 min.-gradient run.

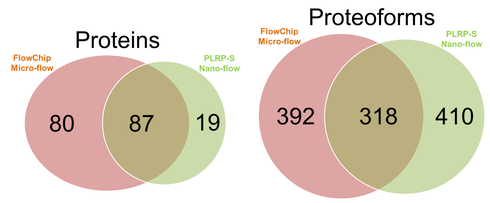

Figure 5. Venn-Diagrams comparing protein (left) and proteoform (right) identifications between micro- and nano-flow rates.

Table 3. Comparison between protein and proteoform coverage at different flow rates.

	Hela lysate	
	Protein IDs	Proteoform IDs
Micro-flow FlowChip 1.5 µL/min	143	657
Nano-flow 0.3 µL/min	92	724

- Proteins from Hela cell lysate were separated in a 90 min.-gradient run.
- Micro and nano-flow rates show comparable results.
- Protein and proteoform were search using TDPortal.

Conclusions

Micro-flow liquid chromatography reveals high-performance in separation and excellent reproducibility for the analysis of peptides, intact proteins, and antibodies:

- Better protein coverage of BSA lysate.
- Increase in detection of unique peptides of Hela lysates.
- High selectivity for the separation of LC, Fd and Fc of digested and reduced SiLu antibody.
- High selectivity for the separation of light chain and heavy chain of reduced SiLu antibody.
- Better peak shape and resolution of top-down standard proteins.
- Protein and proteoform coverage is comparable to those obtained at nano-flow rate for Hela lysates.

Acknowledgments

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