Cutting-edge nanoLC column technology and its analytical capabilities in advanced tandem mass spectrometry proteomics

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Overview

Nanoscale liquid chromatography-mass spectrometry (nanoLC-MS) has been proven to be the most powerful tool for characterization of complex mixtures such as targets of Omics researches. For proteomics, cutting-edge nanoLC-MS, enables measuring >6,500 proteins and >55,000 peptides (bottom-up) and characterizing >10,000 proteoforms for >1,500 proteins (top-down) in a single run od a single-dimension experiment, while the sensitivity allows for performing large-scale single cell proteomics.

Introduction

NanoLC implemented prior to ESI-MS functions focusing molecules from a solution into narrow zones (peaks) to achieve highest MS signals for individual components. Proteomics data quality, quantitated by the number of identifications, is controlled by nanoLC performance when a specific mass spectrometer is applied for data acquisition. Acquiring best datasets requires optimal formats of nanoLC for different proteomic tasks. This presentation demonstrates coverage and sensitivity achievable from cutting-edge nanoLC-MS platforms for situations of most bottom-up and top-down proteomics applications.

Methods

The nanoLC system previously reported (ref 1) was applied for evaluation of various separation columns.

Packed capillary columns were manufactured according to the procedure previously reported (ref 2); the integrated columns were made using the method as reported (ref 3).

Thermo Exactive MS was used for evaluation of separation quality, Orbitrap Fusion Lumos for sequencing of peptides, and Elite for sequencing of proteoforms.

MSGF+ was used for peptide assignment and MSPathFinder for protein assignment (with 1% FDR cutoff).

The quality control samples used at PNNL, including mouse cell lysate for top-down, mouse cell lysate tryptic digestion for bottom-up, and MCF-7 for phosphopeptides, were used for investigations presented here.

Acknowledgements

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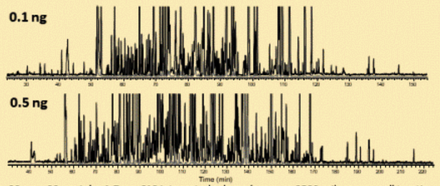
References

1. Shen Y., et. al. *Anal. Chem.* 77, 3090–3100 (2005).
2. Shen Y., et. al. *Anal. Chem.* 74, 4235–4249 (2002).
3. Shen Y., et. al. *Anal. Chem.* 77, 6692–6701 (2005).
4. Zhu Y., et. al. *Nat Commun.* doi:10.1038/s41467-018-03367-w

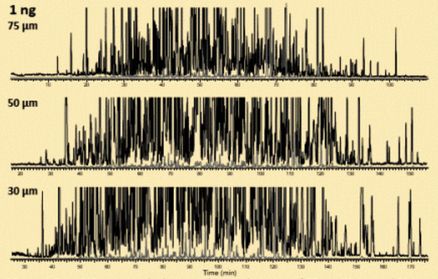
Results

Ultra-sensitive nanoLC for analysis of 0.1–1 ng samples

(Single cell proteomics and proteomics for <10 mammalian cells)

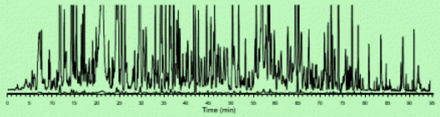


20 cm x 20 μm i.d. x 1,7 μm C18 integrated column (pressure 2500 psi); mouse cell tryptic digest; Exactive MS.

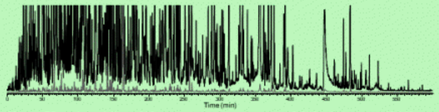


25 cm x 1.7 μm C18 integrated columns with inner diameters of 75 μm, 50 μm, and 30 μm, respectively; mouse cell tryptic digest sample; Exactive MS.

Sensitive and broad analysis of phosphopeptides

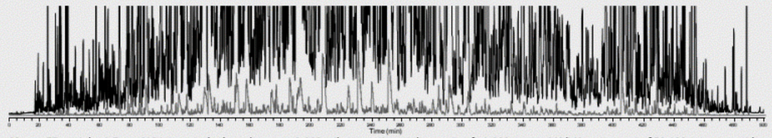


20 cm x 75 μm i.d. x 1.7 μm C18 integrated column (pressure 2,500 psi); Lumos MS/MS assignment of 5,045 phosphopeptides (FDR < 1%) and 2,183 proteins for 10 ng MCF7 phosphoproteomic sample.

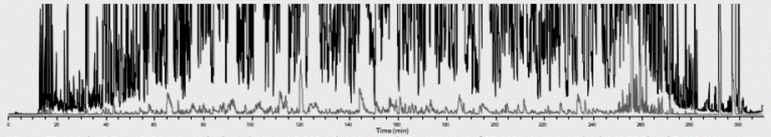


60 cm x 75 μm i.d. x 1.7 μm C18 separated column (pressure 8,000 psi); Lumos; MS/MS assignment of 20,288 phosphopeptides (FDR < 1%) and 4,700 proteins for 300 ng MCF7 phosphoproteomic sample.

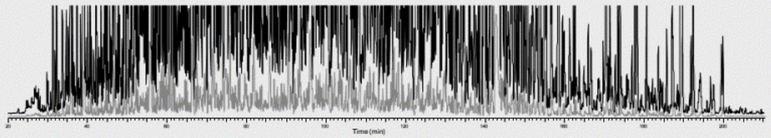
High-resolution long column nanoLC and MS/MS assignment of >55,000 peptides and >6000 proteins



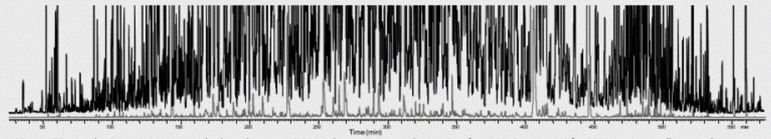
60 cm x 75 μm i.d. x 1.7 μm C18 integrated column (pressure 8,000 psi); separation peak capacity of >1,000; Lumos MS/MS assignment of 58,130 unique peptides (at 1%FDR) and 7,788 unique proteins from 500 ng of mouse cell tryptic digest.



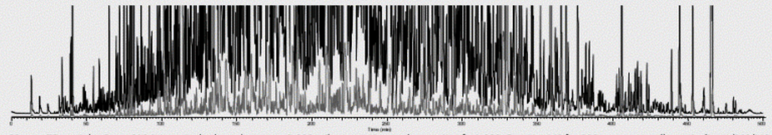
40 cm x 75 μm i.d. x 1.7 μm C18 integrated column (pressure 5,000 psi); Lumos MS/MS assignment of 57,410 unique peptides (at 1%FDR) and 7,403 unique proteins from 500 ng of mouse cell tryptic digest.



30 cm x 75 μm i.d. x 1.7 μm C18 integrated column (pressure 3,900 psi); Exactive MS for 100 ng mouse cell tryptic digest (MS/MS performance not evaluated yet).

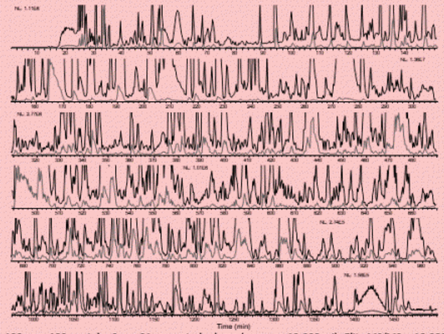


120 cm x 75 μm i.d. x 3 μm C18 integrated column (pressure 8,000 psi); separation peak capacity of >1,000; Exactive MS for 300 ng mouse cell tryptic digest (MS/MS performance not evaluated yet).

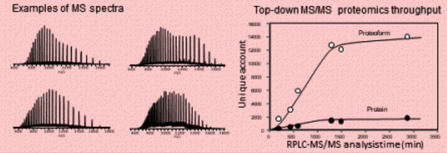


60 cm x 75 μm i.d. x 3 μm C18 integrated column (pressure 8,000 psi); separation peak capacity of >1,000; Exactive MS for 300 ng mouse cell tryptic digest (MS/MS performance not evaluated yet).

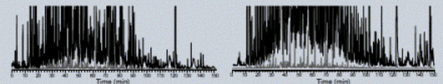
High-resolution top-down nanoLC-MS/MS of proteoforms



120 cm x 100 μm i.d. x 3 μm C4 separated column (pressure 12,000 psi); Elite MS/MS; >10,000 proteoforms are assigned (at 1%FDR) for >1,500 proteins from 5 μg of mouse cell lysate.

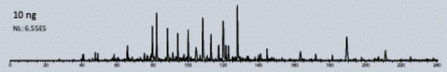


Capability of quality 75 μm i.d. nanoLC columns operated at moderate pressures (used for typical 2D analysis)

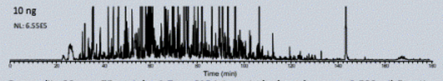


20 cm x 75 μm i.d. x 1.7 μm C18 integrated column (2,500 psi); Exactive; 10-ng mouse cell lysate tryptic digest sample.

20 cm x 75 μm i.d. x 1.7 μm C18 integrated column (2,500 psi); Lumos MS/MS; 18,246 peptides and 3,997 proteins; 10-ng sample.



Waters ACQUITY 20 cm x 75 μm i.d. x 1.7 μm C18 column (pressure 7,500 psi)–Exactive



Our quality 30 cm x 75 μm i.d. x 1.7 μm C18 integrated column (pressure 3,500 psi)-Exactive

Conclusions

1. The ultra-sensitive 20-30 μm i.d. nanoLC columns coupled to Exactive generate dense/intense MS peaks for 0.1-1 ng mammalian bottom-up proteomic samples. Sample sizes of 0.1-1 ng are equivalent to the protein contents of 1-10 mammalian cells (~10 μm size) or <0.05 mm tissues. Compared to our MS and MS/MS data obtained for an individual and 10 cells (not shown), we estimate that such sensitivity can lead to assignment of 1,000-4,000 proteins when sensitive Lumos (or Q Exactive) is applied. Special sample manipulation is necessitated for such tiny samples. NanoPots device designed for processing of 10-100 cells[4] may be a choice, but needs further reduction of sample loss for achieving a broad coverage proteomic analysis of 1-10 cells.
2. High-resolution nanoLC can separate proteoforms with peak capacity of up to 1000, allowing top-down MS/MS characterization of >10,000 proteoforms of >1,500 proteins in a single run of an one-dimension experiment. Further enlarging proteome coverage needs fractionating the sample prior to the high-resolution nanoLC-MS/MS analysis due to limited effectiveness of mass spectrometry for protein detection/sequencing. Finding significances of proteoforms functioning in a specific biological/biomedical process becomes a critical importance for fastening next stage development of top-down proteomics.
3. High-resolution nanoLC in combination with sensitive and fast Lumos MS/MS (or Q Exactive) enables assignment of >6,500 proteins and >55,000 peptides (at 1% FDR) for a ≤500-ng proteomic sample in a single 300-500 min run. With such high proteome coverage, this single-dimension analysis approach favors simplifying proteomic analysis complexity, improving analysis throughput, better quantitation, and especially, the sensitivity for analysis of small sizes of samples (e.g., a few cells or tiny tissues), as implement of any extra dimension separation(s) will result in more or less sample losses.
4. The quality of column greatly affects nanoLC separation peak shapes and mass spectrometry detection sensitivity. High-quality moderate length 75 μm i.d. columns enable nanoLC-MS/MS to assign ~20,000 peptides and ~4000 proteins for a 10-ng sample in 2-3 hours, desirable for use in a conventional two-dimensional bottom-up proteomic analysis.
5. High-quality 75 μm i.d. column nanoLC-MS/MS has a capability for assignment of >20,000 phosphopeptides from >4,500 proteins for a 300-ng sample and >5,000 phosphopeptides from >2,000 proteins for a 10-ng sample. Ultra-sensitive nanoLC columns are preferred for phospho-proteomic analysis, as phospho-subproteome may have a larger dynamic range than a global proteome as well as the sample size available is often limited.

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