ionopticks

APPLICATION NOTE

Maximize protein identifications from your Q-Exactive using Aurora Series columns

INTRODUCTION

Celebrating over a decade of excellence, the Thermo Fisher Q-Exactive Mass Spectrometer series has been a staple proteomics platform found in labs worldwide. Conventional proteomics workflows on the Q-Exactive relied on nanoflow-columns which failed to demonstrate the full-potential of this instrument. IonOpticks columns leverage ease of use, high reproducibility and narrow peak widths to maximise protein identifications across different applications. We combined the IonOpticks Aurora Ultimate TS column with the Q-Exactive Plus to elevate the performance of this classic instrument and demonstrate the result of this perfect pairing across a broad range of sample loads, gradients and search algorithms.

Three mass spectrometry methods alongside three analytical platforms were evaluated in this study. The methods include Narrow Window Acquisition (NWA), Wide Window Acquisition (WWA), and Data-Independent Analysis (DIA). The data was processed using three different search algorithms: MaxQuant, Chimerys/Proteome Discoverer, and DIA-NN. This analysis demonstrated that we were able to identify more than 6100 unique proteins and up to 47,000 unique peptides from a single 200 ng Hela injection. This was achieved whilst retaining <9 % CVs, <7 sec FWHM peak widths and stable retention times for over 100 runs.

The combination of IonOpticks columns with the Q-Exactive series of mass spectrometers equips scientists with the capability to identify a broader array of peptides and proteins from their precious samples, ensuring consistent results across various experiments and enabling a deeper understanding of biological systems.

6,100+ proteins identified from a single HeLa injection.

Maximum proteins identifications across a broad range of sample loads

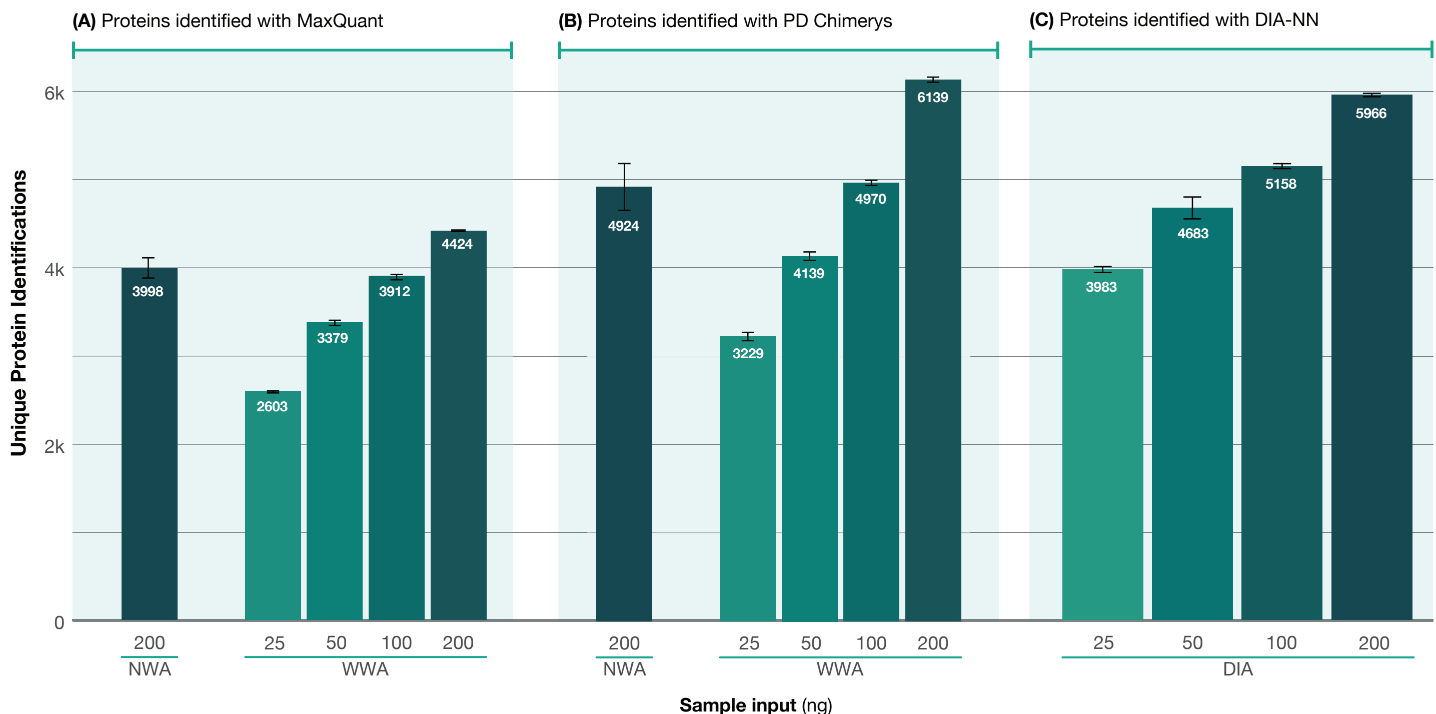


Figure 1: Unique protein identifications across a broad range of sample loads and search algorithms. A dilution series of a HeLa tryptic digest (25 ng – 200 ng) was separated on an Aurora Ultimate 25 cm x 75 µm column on a 90 min gradient using a Thermo Vanquish Neo UHPLC and Q-Exactive Plus MS, n = 6. Match-Between-Runs was not selected in any search. **(A)** Samples were analysed using either NWA or WWA mass spectrometry acquisition methods and results searched using MaxQuant. **(B)** The same raw files from (A) were searched using the Chimerys algorithm embedded within Proteome Discoverer 3.1. **(C)** Samples were analysed using a DIA mass spectrometry acquisition method and results searched using DIA-NN 1.81.

Narrow peak widths

(A) FWHM at different sample inputs and gradient lengths

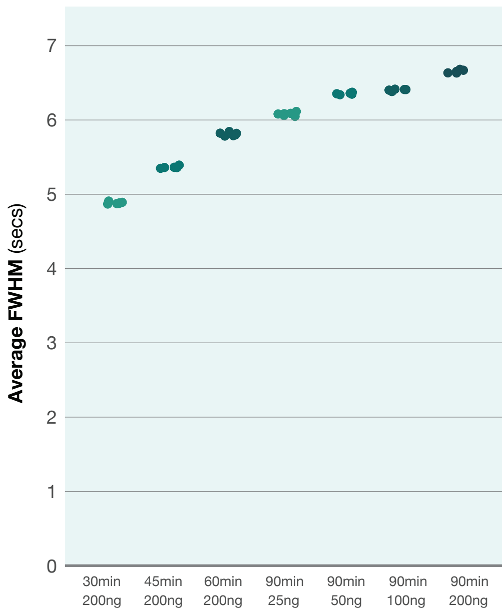


Figure 2: **Peak widths across different gradient lengths and sample loads.** Average Full Width at Half Maximum (FWHM) for all identified peptides from HeLa Tryptic Digest injections on an Aurora Ultimate 25 cm x 75 µm column. Samples were run on a Thermo Vanquish Neo UHPLC and Q-Exactive Plus MS, using WWA. Data analysed using MaxQuant, n=6.

Maximum reproducibility

(B) CV% across different sample inputs and gradient lengths

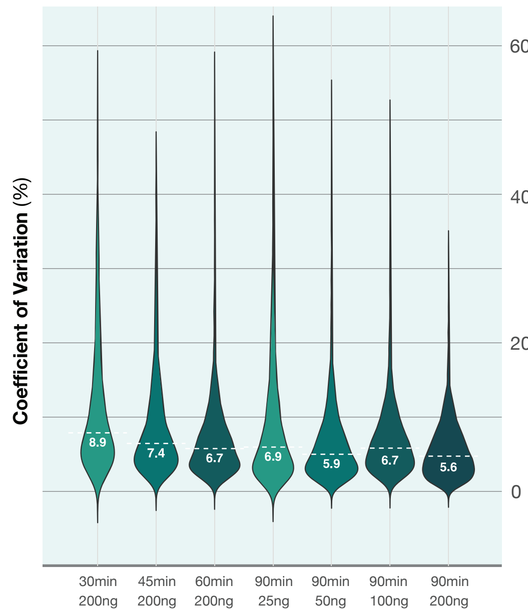


Figure 3: **Low CVs across different gradient lengths and sample loads.** Violin plot of Coefficient of Variation for all identified protein intensities from HeLa Tryptic Digest injections on an Aurora Ultimate 25 cm x 75 µm column. Samples were run on a Thermo Vanquish Neo UHPLC and Q-Exactive Plus MS, using WWA. Dashed line represents the median value for each sample. Data analysed using MaxQuant LFQ, n=6.

Up to
47k
peptides identified from 200 ng

Less than
7sec
FWHM
peak widths

>0.9
Pearson correlation of protein intensities across 100+ runs

Maximum protein identifications across a broad range of gradient lengths

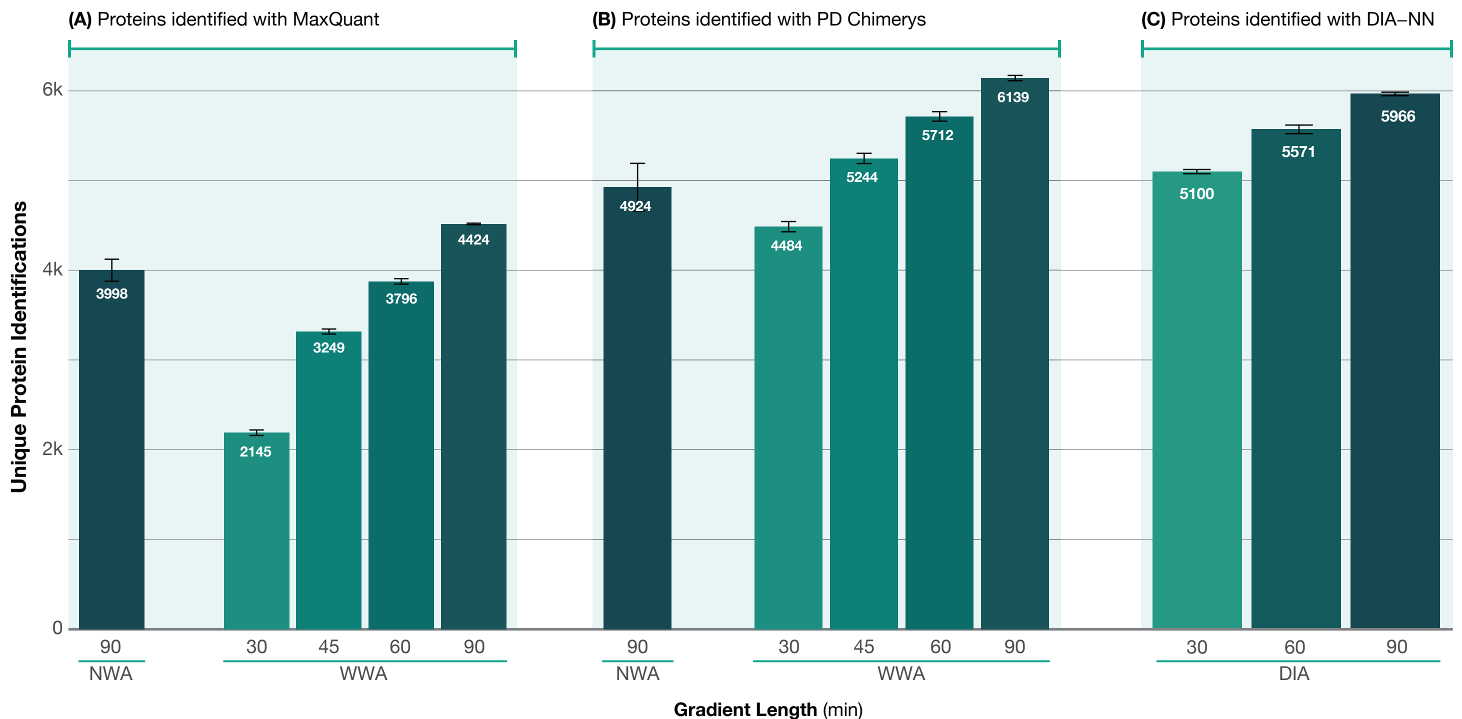


Figure 4: **Unique protein identifications across a range of gradient lengths and search algorithms.** 200 ng of a HeLa tryptic digest was separated across a range of gradient lengths (30 min – 90 min) on an Aurora Ultimate 25 cm x 75 µm column using a Thermo Vanquish Neo UHPLC and Q-Exactive Plus MS, n = 6. Match-Between-Runs was not selected in any search. **(A)** Samples were analysed using either NWA or WWA mass spectrometry acquisition methods and results searched using MaxQuant. **(B)** The same raw files from (A) were searched using the Chimerys algorithm embedded within Proteome Discoverer 3.1. **(C)** Samples were analysed using a DIA mass spectrometry acquisition method and results searched using DIA-NN 1.81.

Maximum peptide identifications across a broad range of sample loads and gradients

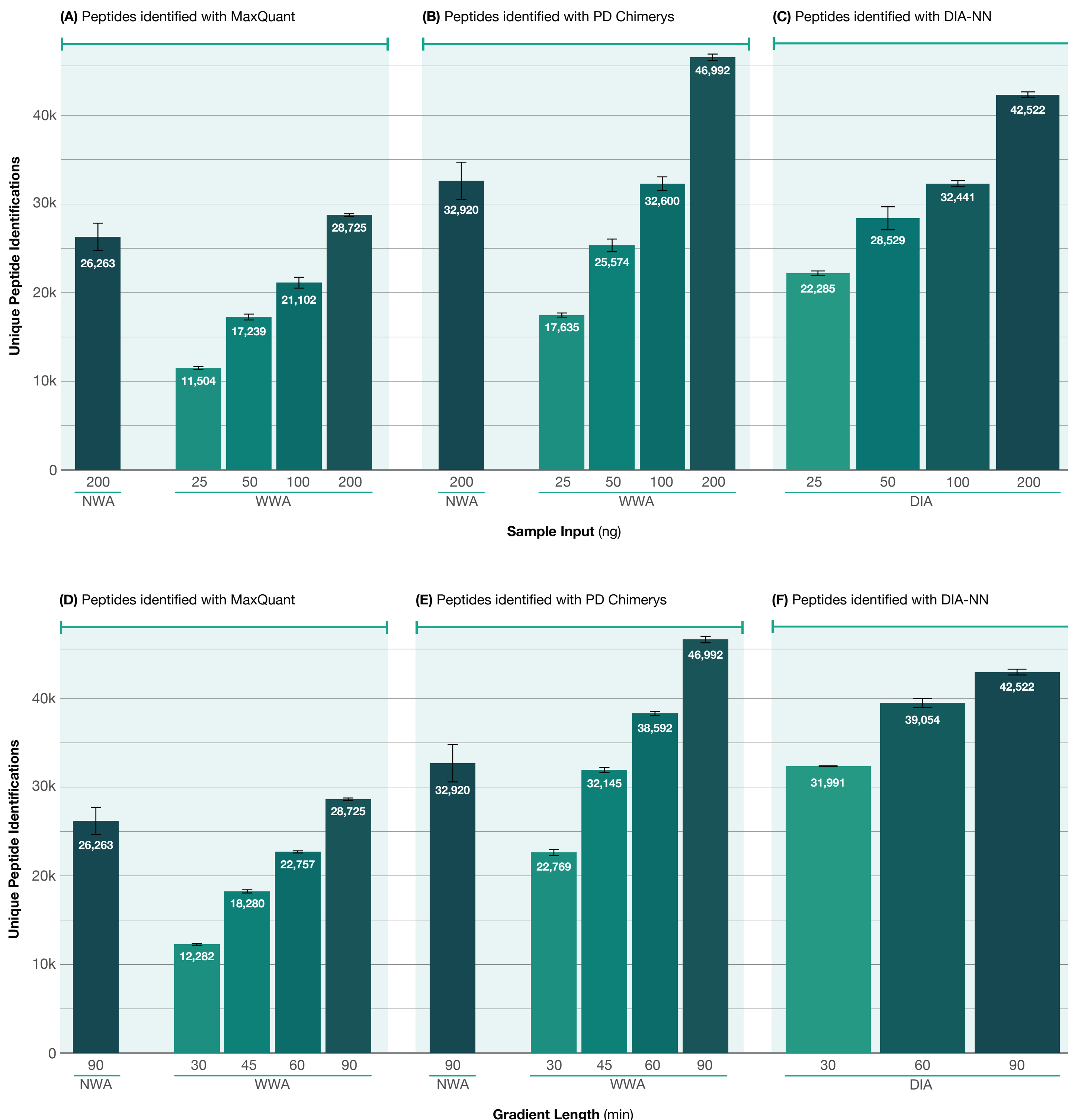
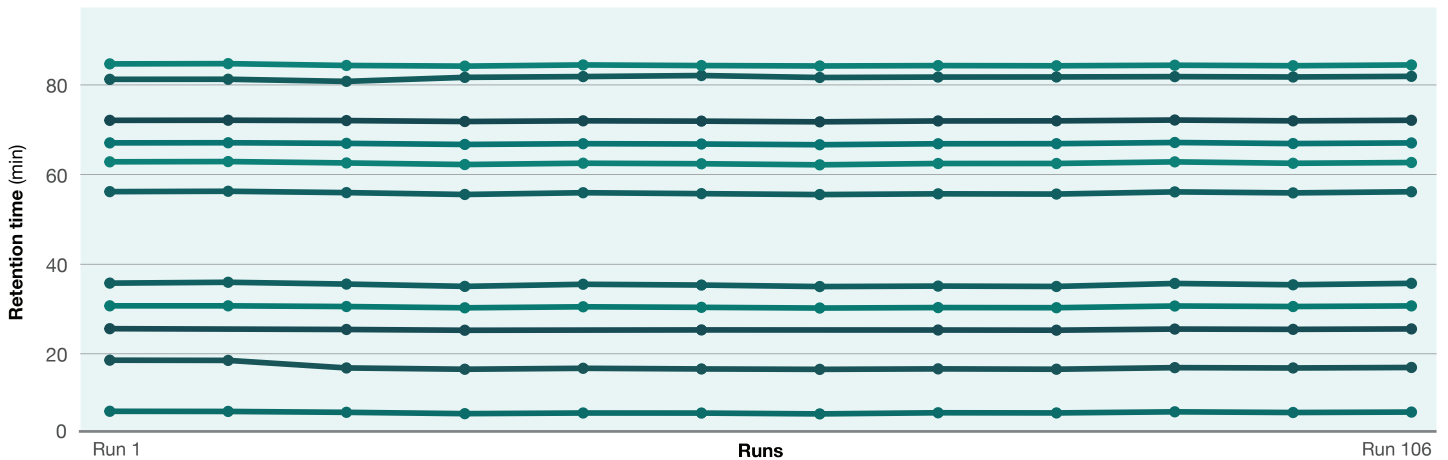


Figure 5: Unique peptide identifications across a broad range of sample loads, gradients, and search algorithms. (A) A dilution series of a HeLa tryptic digest (25 ng – 200 ng) was separated on an Aurora Ultimate 25 cm x 75 µm column on a 90 min gradient using a Thermo Vanquish Neo UHPLC and Q-Exactive Plus MS, n = 6. Match-Between-Runs was not selected in any search. Samples were analysed using either NWA or WWA mass spectrometry acquisition methods and results searched using MaxQuant. **(B)** The same samples and raw files from (A) were searched using the Chimerys algorithm embedded within Proteome Discoverer 3.1. **(C)** Samples were analysed using a DIA mass spectrometry acquisition method and results searched using DIA-NN 1.81. **(D)** 200ng of a HeLa tryptic digest was separated across a range of gradient lengths (30 min – 90 min) on an Aurora Ultimate 25 cm x 75 µm column using a Thermo Vanquish Neo UHPLC and Q-Exactive Plus MS, n = 6. Match-Between-Runs was not selected in any search. Samples were analysed using either NWA or WWA mass spectrometry acquisition methods and results searched using MaxQuant. **(E)** The same samples and raw files from (D) were searched using the Chimerys algorithm embedded within Proteome Discoverer 3.1. **(F)** Samples were analysed using a DIA mass spectrometry acquisition method and results searched using DIA-NN 1.81.

High reproducibility across 100+ sample runs

(A) Retention time across 12 days and 106 runs



(B) Pearson Correlation Matrix across sample runs

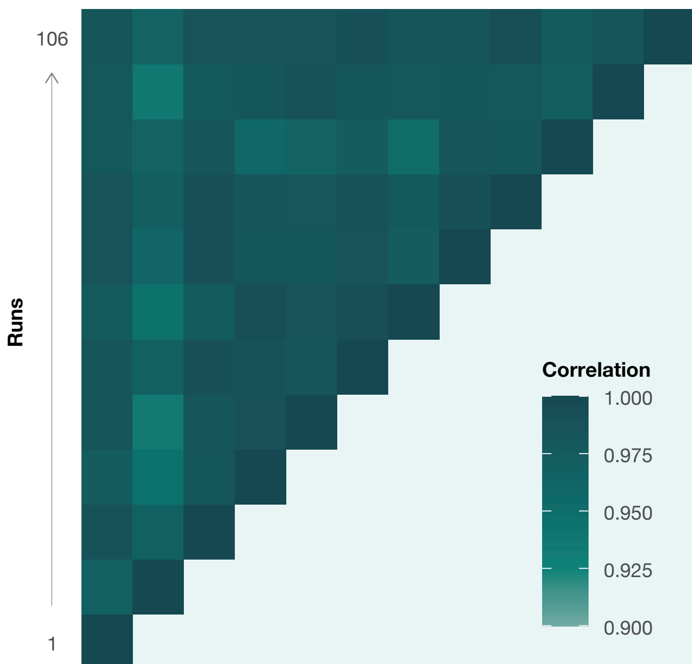


Figure 5: Stable peptide retention times and protein intensities across 100+ sample runs. 12 injections of 200ng of a HeLa Tryptic digest using a 90 min gradient, interspaced across 100+ sample injections were acquired using NWA on a Thermo Vanquish Neo UHPLC and Q-Exactive Plus MS. Raw data was analysed using MaxQuant LFQ. **(A)** 10 peptides were selected from the HeLa Tryptic digest and their retention time assessed across the acquisition period. No significant deviation of retention times was observed. **(B)** A Pearson correlation matrix of all quantified protein intensities from the 12 injections was calculated.

METHODS

Hela Tryptic digest was reconstituted in 2% ACN, 0.1% FA in water. Sample was injected using a Thermo Fisher Scientific Vanquish Neo LC. Gradients were run at 400 nL/min over gradients lengths of 30, 45, 60 or 90min, at sample inputs of either 25, 50, 100 or 200ng. Buffer A: 99.9 % water, 0.1 % Formic Acid. Buffer B: 99.9% ACN, 0.1% water. The Vanquish Neo was connected to an IonOpticks Aurora Ultimate TS column (25 cm x 75 um). Acquisition was performed using a Thermo Fisher Scientific Q-Exactive Plus MS. Data-Dependant methods were acquired with either narrow window acquisition (1.5 Da) or wide window acquisition (4 Da). Both methods used MS1 resolution 70,000 across a m/z range of 400 - 2000, and a MS2 resolution of 17,500 across 200-2000 m/z. Top 20 MS spectrum were selected for every MS1 scan. HCD fragmentation energy of NCE 27 and spray ionisation of 1.8kV was used. DIA methods used 20 m/z acquisition windows across an MS1 range of 490 - 910 m/z. Three data analysis platforms were utilised, MaxQuant 1.6.17, Proteome Discoverer 3.1 with Chimerys and DIA-NN 1.81. MaxQuant searches were performed using LFQ and fast normalisation without match between runs. Proteome Discoverer used default Chimerys processing workflows and basic annotation template for the consensus workflow. DIA-NN 1.81 library free searching used default parameters for the acquired MS1 mass range (490-910). Match between runs was not selected. All three platforms were used searching against Uniprot human database. All Data was filtered to 1% False Discovery Rate.

CONCLUSION

The combination of the IonOpticks Aurora Ultimate column and Thermo Q-Exactive Plus enabled maximum protein and peptide identifications from a broad range of sample loads, gradients and analysis software suites. IonOpticks packed emitter columns allow scientists to unlock the full potential of their mass spectrometer.

ACKNOWLEDGEMENTS

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ABOUT IONOPTICKS

IonOpticks produces high-performance chromatography solutions for the global research community. We specialise in the development and manufacture of columns for analytical applications in liquid chromatography with mass spectrometry (LC-MS) and high-end proteomics. Our highly reproducible methods provide a unique ability to enhance the sensitivity of mass spectrometry sample analysis, enabling scientists and clinicians to discover more from their samples. Our team are experts in a broad array of LC-MS platform technologies and are driven by the need to improve chromatographic performance in order to achieve data quality and deep proteome coverage on a whole new scale.