Flash Sponsored Talks BePAc2018

Bruker: Maximized throughput and analytical depth for clinical shotgun proteomics research using PASEF on a TIMS equipped QTOF and a novel LC system

Shotgun Proteomics has proven to be an extremely valuable tool for generating detailed information on protein presence, regulation or modification in a given biological sample. The use as a biomarker candidate generation pipeline has been less effective, as one of the challenges raised by clinical research is the huge genetic and phenotypic variability that can be observed among clinical samples of human origin. This variability is the reason why very large sample cohorts have to be analysed to obtain a statistical power that is compatible with the aim of clinical proteomics discovery approaches.

In this presentation, we describe how a TIMS equipped QTOF operated in PASEF acquisition mode and connected to a novel LC system allows to reach a combination of throughput, analytical depth, robustness and sensitivity that is compatible with the requirements of clinical proteomics discovery.

The operation mode of the Tims-PASEF as well as the one of the Evosep LC system will be described, and the first results will be presented. A repeated injection of 50ng of an Hela Cell digest separated with a 5,6 min gradient, allowing a throughput of 200 samples/day, allowed for the reproducible identification of more than 1400 protein groups, among which 1000 could be reproducibly quantified. On a 100 measurements injection test, all analysis could be correlated with very high R2 values, underlining that the analyser's performances were maintained with these very short gradients.

PharmaFluidics: Maximize the output of routine proteome analyses by using a 50 cm long microfabricated nano LC column

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As an alternative to the conventional packed bed nano LC columns that are frequently used in bottom-up proteomics research, PharmaFluidics offers micromachined nano LC chip columns known as micro pillar array columns (µPAC™). The inherent high permeability and low 'on-column' dispersion obtained by the perfect order of the separation bed makes µPAC™ based chromatography unique in its kind. The peak dispersion originating from heterogeneous flow paths in the separation bed is eliminated (no A-term contributions) and therefore components remain much more concentrated during separation resulting in unprecedented separation performance (1). The freestanding nature of the pillars also leads to much lower backpressure allowing an high operational flow rate flexibility with exceptional peak capacities (2).

Complementary to its landmark 200 cm long column which is ideally suited to perform comprehensive proteome research, a 50 cm long µPAC™ column is now available which can be used in a more routine research setting. With an internal volume of 3 µL, this column is perfectly suited to perform high throughput analyses with shorter gradient solvent times (30, 60 and 90 minute gradients) and it can be used over a wide range of flow rates, between 100 and 2000 nL/min. Recently performed experiments with 500 ng of HeLa cell digest indicate that an increase in protein identifications up to 50% and a gain of 70% in peptide identifications can be achieved when comparing the 50 cm µPAC™ column to the current state-of-the-art in packed bed columns. The conventional packed bed columns (2 different vendors) used for this benchmarking experiment were 15 cm in length and were packed with sub 2 µm porous silica particles. LC pump pressures needed to operate these classical columns at a flow rate of 300 nL/min range between 200 and 300 bar, whereas only 40 bar was need to operate the 50 cm µPAC™ column at the same conditions.

(1) W. De Malsche, H. Gardeniers, G. Desmet, Experimental Study of Porous Silicon Shell Pillars under Retentive Conditions, *Anal. Chem.* 80 (2008) 5391-5400.
(2) W. De Malsche, J. Op De Beeck, S. De Bruyne, H. Gardeniers, G. Desmet, Realization of 1 × 10E6 Theoretical Plates in Liquid Chromatography Using Very Long Pillar Array Columns, *Anal. Chem.*

Thermo Scientific: Advances in Orbitrap Instrumentation for Native Top-Down Analysis of Non-Covalent Protein Complexes

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To understand protein function and mechanism of action, it is essential to determine protein complex assembly and structure. Thermo Fisher Scientific leads the way in accelerating protein structure-function studies with its Integrative Structural Biology solutions. Thermo Scientific Orbitrap™-based mass spectrometers enable characterization of complex and dynamic structure-function relationships. Here we will share new advancements in Orbitrap™ instrumentation for structural analysis of large macromolecular complexes using native MS.

Native mass spectrometry has emerged as a powerful technique to study protein-ligand interactions and elucidate the structure of macromolecular assemblies, including both soluble and membrane protein complexes. The approach relies on maintaining a biomolecule's natural folded state and associated non-covalent interactions for MS analysis.

The Thermo Scientific Q Exactive UHMR hybrid Quadrupole-Orbitrap™ mass spectrometer combines in-source trapping, high performance quadrupole precursor ion selection, a higher-energy collisional dissociation (HCD) cell, and a high-resolution, accurate-mass (HRAM) Orbitrap™ mass analyzer, with optimized RF voltages for improved high mass ion transmission to provide unrivaled sensitivity and resolution in the ultra-high mass range.

In this work we demonstrate the excellent performance of the new Q Exactive UHMR mass spectrometer for structural characterization by top-down pseudo-MS^3 of homomeric and heteromeric protein assemblies using GroEL, rabbit 20S proteasome and AmtB membrane protein complex as model systems.

Waters: Use of a novel DIA acquisition for enhanced throughput analysis with an exploratory targeted reagent strategy for Proteomics in Clinical Research

David Heywood
Senior Manager Omics Business Development, Waters Corporation

Success in biomarker studies in clinical research requires analysis of samples from large human cohorts. As these study sizes grow increased strain is placed on the laboratory and indeed the analytical approach. In order to be successful, researchers are faced with the challenge of balancing proteomic coverage with accurate, reproducible and robust LC-MS measurements. In this presentation we will describe how the combination of SONAR, a new data independent acquisition (DIA) mode, rapid UPLC separations and a comprehensive stable isotope labeled reagent kit addresses this challenging scenario.

Sciex: Accelerating Proteomics on the TripleTOF® 6600 (Up to 100 Samples per Day)

Zuzana Demianova[a]; Nick Morrice[b], Xiaolei Lv[c], Christie Hunter[d]

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Understanding the impact of gradient length on proteins quantified will provide researchers workflow options depending on the sample complexity and the size of the study (# of samples to be run) when performing microflow SWATH acquisition studies.

The combination of microflow LC with SWATH Acquisition for large scale discovery and quantitative proteomics studies is becoming increasingly more widespread, due to the improved robustness and throughput obtained relative to the traditional nanoflow LC approach. Combined with MS/MS acquisition rates as fast as 66 Hz on the TripleTOF® 6600 System, good protein identification results are achieved providing high sample throughput. Using multiple instruments and multiple complex matrices, it was observed that the number of proteins identified for even the fastest gradients was still over 1000 with 1% FDR and over ~1500 proteins were quantified with 1%FDR and CV>20% for multiple complex matrices from 1 µg protein load.

The impact of library size was also tested on the accelerated gradients and provided improved results as expected. With the Pan Human Library on a 1ug load of HEK lysate, ~2100 and ~3400 proteins were quantified with the 5 and 10 min gradients respectively.

The combination of microflow LC with the high MS/MS acquisition rates of the TripleTOF® 6600 system enables high throughput yet comprehensive analysis for proteomics samples, at rates approaching 100 samples per day.