Proteome Quality Control Addressing Qualitative and Quantitative Needs for Trapped Ion Mobility Spectrometry and PASEF

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Introduction

In this study we utilize stable isotope labeled peptides and spiking them into a human leukemia cell line. We explore sample loads, gradient lengths and software approaches to simultaneously optimize qualitative and quantitative qualification in a expedient manner (<30 min) on a mass spectrometer with maximum sequencing speed, true CCS determination and sensitivity (timsTOF Pro).

Methods

K562 digested protein was reconstituted to 100 ng/uL. Fifteen yeast peptides (Peptide Retention Time Calibration mixture) and a series of 35 peptide isotopologues (7x5) at 5 orders of magnitude different concentrations, between 25 fmol (heaviest) to 8 amol (lightest), were spiked into the cell lysate. Three different concentrations of Cell lysate/spiked peptides (25ng/12.5fmol, 50ng/25fmol, and 100ng/50fmol) were loaded into a Bruker nanoElute in triplicate, at a flow rate of 400 nL/min, before being reverse phase separated using a 25 cm Ionopticks Odyssey column. 15 and 30 min gradients were tested where peptide ions were ionized into the timsTOF Pro mass spectrometer and data acquisition was performed in PASEF mode. The tims device was set to accumulate and elute at 100ms, resulting in 100% duty cycle. Ion mobility was determined to be between 0.70 and 1.50 V·s/cm^2 after preliminary SIL testing. Data analysis was performed on TDF files using PEAKS X, Byonic and Compass DataAnalysis.

Table 1. Preliminary Pierce Retention Time Calibration Mixture run @ 15 min gradient with added trapped ion mobility values

Peptide Sequence	Mass (M)	Observed *m/z* z = 2	Hydrophobicity Factor	Avg. Peak Width FWHM (Sec)	Avg. RT	RSD %	Avg Ion Mobility
SSAAPPPPPR	985.522	493.7683	7.56	4.27	5.9	0.00	0.75-0.77
GISNEGQNASIK	1224.6189	613.3167	15.5	1.87	6.4	0.00	0.85-0.87
HVLTSIGEK	990.5589	496.2867	15.52	2.20	6.5	0.00	0.80-0.82
DIPVPKPK	900.5524	451.2834	17.65	6.70	8.6	0.67	0.77-0.79
IGDYAGIK	843.4582	422.7363	19.15	2.70	9.0	0.00	0.74-0.76
TASEFDSAIAQDK	1389.6503	695.8324	25.88	2.87	10.9	0.00	0.92-0.96
SAAGAFGPELSR	1171.5861	586.8003	25.24	4.37	11.6	0.50	0.83-0.86
ELGQSGVDTYLQTK	1545.7766	773.8955	28.37	2.47	13.2	0.00	0.99-1.03
GLILVGGVGTR	1114.6374	558.3259	32.18	2.67	15.0	0.00	0.87-0.89
SFANQPLEVVYSK	1488.7704	745.3924	34.96	2.70	15.1	0.38	0.96-0.99
GILFVGSGVSGGEEGAR	1600.8084	801.4115	34.5	2.73	15.4	0.38	0.97-1.02
LTILEELR	995.589	498.8018	37.3	2.47	16.5	0.00	0.81-0.83
NGFILDGFPR	1144.5905	573.3025	40.42	3.70	17.6	0.00	0.81-0.87
ELASGLSFPVGFK	1358.7326	680.3735	41.18	2.27	18.1	0.00	0.90-0.96
LSSEAPALFQFDLK	1572.8279	787.4212	46.66	1.60	18.6	0.00	0.99-1.02

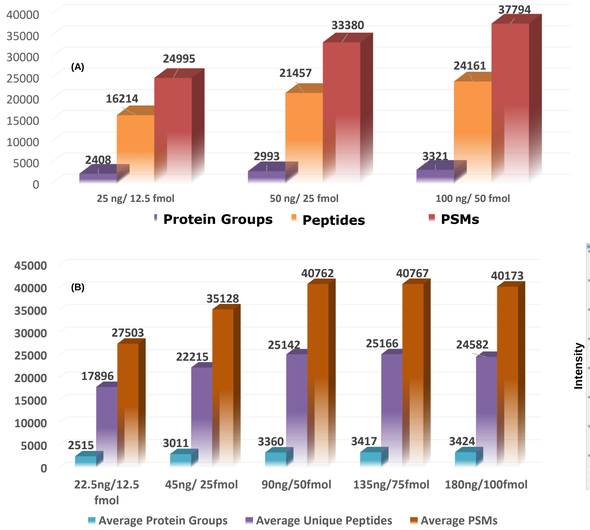

Fig.1 Average protein groups, peptides and PSMs for triplicate injections of (A) K562 spiked with PRTC and (B) K562 spiked with Pierce 7x5 using a **15 min** gradient on the timsTOF Pro. The 7x5 concentration represents the heaviest peptide concentration

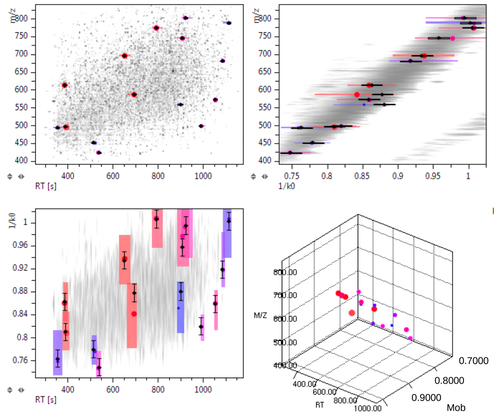

Fig.2 Observed elution of heaviest yeast AQUA peptide at concentration 12.5 fmol (heaviest of the AQUA peptides) using a 15 min gradient on the timsTOF Pro. Figures were created using prototype Feature Finder and Feature Viewer software (A) RT vs m/z (B) Ion Mobility vs m/z (C) RT vs 1/k0 (D) Ion Mobility vs RT vs M/z are displayed

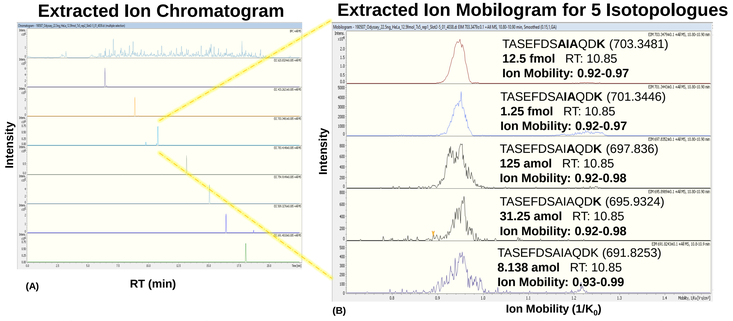

Fig.3 (A) Observed elution of heaviest yeast AQUA peptide at concentration 12.5 fmol (heaviest of the AQUA peptides) using a 15 min gradient on the timsTOF Pro. (B) Extracted ion mobilogram (EIM) for five isotopologue versions containing no heavy, 1 heavy, 2 heavy, 3 heavy, and 4 heavy isotope-labeled amino acids. Isotopologues are chromatographically indistinguishable; however, since they differ in mass, they can be resolved by the EIM.

Data

We optimized experimental conditions to achieve peak instrument performance in under an hour (total run time). Sample and instrument parameters were tested to develop a fast, reproducible and robust LC-MS method. Two matrices: K562 spiked with 100 fmol/uL (heaviest peptide) and K562 spiked with PRTC were selected after preliminary studies monitoring protein/peptide/PSM identification values, observed isotopologue sensitivity and chromatographic properties (peak shape, FWHM, tailing, etc). Samples were run on the nanoElute HPLC running at 400 nl/min.

Optimized timsTOF Pro settings: Capillary voltage: 1600V, gas flow 3 l/min, gas temp: 180C, imex values were between 0.7 and 1.50 V • s/cm2 (End set to 2.10 V • s/cm2), accumulation time: 100 ms, cycle time approximately 73.0 ms (with 100% duty cycle enabled), 10 PASEF MS/MS acquired at a rate of **12.82 Hz (128.2 Hz) per 0.87 sec cycle time.**

The trapped ion mobility capabilities resulted in determinate collisional cross section (CCS) terms for each of the analytes identified. We have archived the CCS values for all thirty five isotopologues and 15 PRTC (Yeast) peptides, thereby resulting in a third dimension to validate peptide/protein numbers, quantitative response and reproducibility of CCS terms.

Results

In this study we describe mass offset mobility aligned (MOMA), a phrase defining analytes (i.e. SIL peptides) with minor mass-to-charge differences, indistinguishable by retention time or primary sequence, all having the same collisional cross section (ion mobility)

We established ion mobility values for 50 commonly used Yeast standard peptides. When ion mobility and retention time merge with the complexity of a digested lysate spiked with SIL peptides, the result is the most complete approach to monitor both LC and MS instrument sensitivity and performance.

Conclusions

- More than **2500 Protein groups**, **17800 Unique peptides** were identified from 22.5 ng K562 using a **15 min** gradient
- More than **3300 Protein groups**, **25000 Unique peptides** were identified from 90 ng using a **15 min** gradient
- Frame acquisition rate was **>140 Hz**
- To date this is the most complete approach to monitor both LC and MS instrument sensitivity and performance

timsTOF Pro