Data Descriptor

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Deep profiling of proteomics dataset by liquid chromatography/trapped ion mobility spectrometry/tandem mass spectrometry

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Keywords

Collision cross-section (CCS), Trapped ion mobility spectrometry, Peptide ion mobility

Dataset summary

Specific subject area	Ion mobility spectrometry-mass spectrometry for proteomics profiling
Data acquisition	Data-dependent acquisition mode using timsTOF Pro (Bruker)
Dataset repository	jPOST
Dataset identifiers	JPST000959, JPST001017, JPST001176

Abstract

Trapped ion mobility spectrometry (TIMS) has been considered as a promising tool in structural biology, proteomics and many other analytical applications. This dataset consists of cell lysates digested with each of seven proteases (trypsin, LysargiNase, Lys-C, Lys-N, Glu-C, Asp-N, and chymotrypsin), fractionated using SCX-StageTip, and analyzed by nanoLC/TIMS/Q/TOF. The data accompanying this paper have been deposited to jPOST with the identifiers JPST000959, JPST001017 and JPST001176.

1. Materials and Methods

1.1. Samples

HeLa S3 (human cervical adenocarcinoma) cells were cultured in Dulbecco's modified Eagle's medium until 80% confluent in 10 cm diameter dishes. To prepare cell lysates, cells were washed twice with PBS and solubilized in lysis buffer containing protease inhibitor (Sigma-Aldrich, St. Louis, MO), 12 mM sodium deoxycholate (SDC), 12 mM sodium lauroylsarcosinate (SLS), 10 mM tris(2-carboxyethyl)phosphine (TCEP), 40 mM chloroacetamide (CAA) in 100 mM Tris buffer (pH 8.5). Another sample used in this study is *E. coli* K12 strain BW25113 cells grown at 37 °C in Luria-Bertani (LB) culture. The lysate was prepared by centrifugation at 4500 × g for 10 min and resuspended in 10 mL of ice-cold 1 M KCl, 15 mM Tris (pH 7.4). The protease inhibitor AEBSF was added to a final concentration of 10 mM. Proteins were extracted with 12 mM SDC, 12 mM SLS, 50 mM bicarbonate ammonium, reduced with 10 mM dithiothreitol for 30 min at room temperature, and alkylated with 55 mM iodoacetamide for 30 min at room temperature in the dark.

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1.2. Sample pretreatment for MS analysis

Lysates were digested using previously described phase-transfer surfactants (PTS) [1] method with some adjustment. For LysargiNase (Merck, Darmstadt, Germany) digestion [2], protein extract was diluted 10-fold by using 10 mM $CaCl_2$ and digested with LysargiNase (1: 40 w/w) overnight at 37 °C. For other proteases, extracts were diluted 5-fold by with 50 mM ABC and digested overnight at 37 °C using trypsin (1: 40 w/w), Lys-C (1: 20 w/w), Lys-N (1: 50 w/w), Glu-C (1: 20 w/w), Asp-N (1: 40 w/w), chymotrypsin (1: 50 w/w) protease/substrate ratios. After enzymatic digestion, an equal volume of ethyl acetate was added, and the mixture was acidified with 0.5% trifluoroacetic acid (final concentration) according to the PTS protocol. The mixture was shaken for 1 min and centrifuged at 15,700 g for 2 min to separate ethyl acetate phase from the aqueous phase. The latter was collected and desalted by using SDB-StageTips [3]. The amount of peptides was quantified by LC-UV at 214 nm relative to standard BSA tryptic digests and kept at 80% ACN and 0.5% TFA at −20 °C until use.

Digested peptides were fractionated by Strong Cation Exchange StageTip. The preparation of SCX-StageTips were performed in 200-µL tips format as described previously [4]. SCX buffers were made in 15% acetonitrile with stepwise increase of elution buffer strength: F1 - 0.1% TFA; F2 - 1.0% TFA; F3 - 2.0% TFA; F4 - 3.0% TFA; F5 - 3.0% TFA and 100 mM AA; F6 - 3.0% TFA and 500 mM AA and; F7 - 0.1% TFA and 500 mM AA, as described previously [5]. Two technical replicate SCX separations have been done for each digest. Conditioning and equilibration were done through sequential passing 100 µL buffer and centrifugation at 1000 × g for 1 min of the following buffers: methanol, F7, F5 and F1. Twenty µg of digests from HeLa cell lysate were loaded into the SCX-StageTip, spun at 1000 × g for 1 min and the eluate was collected as flow-through (FT). The bound peptides were eluted with 100 µL of F1 by centrifugation at 1000 × g for 1 min. Subsequent fractions were collected using 100 µL of SCX buffers F2 to F7. F5−F7 were lyophilized, resuspended in 50 µL of 0.1% TFA and desalted by SDB-StageTips.

1.3. MS analysis

NanoLC/MS/MS analyses were performed using a hybrid ESI/TIMS/Q/TOF mass spectrometer (timsTOF Pro, Bruker, Bremen, Germany), which was connected to an Ultimate 3000 pump (Thermo Fisher Scientific, Germering, Germany) and an HTC-PAL autosampler (CTC Analytics, Zwingen, Switzerland). Peptides were separated at 50 °C using 150 mm length × 100 µm ID capillary column with 6 µm ID ESI tip, packed with Reprosil-Pur 120 C18−AQ 3 µm particles (Dr. Maisch, Ammerbuch, Germany). The injection volume was 5 µL and the flow rate was 500 nL/min. The mobile phases consisted of (A) 0.5% acetic acid and (B) 0.5% acetic acid and 80% ACN. A two-step linear gradient of 5−40% B in 45 min, 40−99% B in 1 min, keeping at 99% B for 5 min was employed.

The timsTOF Pro mass spectrometer was operated in PASEF mode [6]. Two methods were applied in IMS separation. Method 1 was applied for covering singly and multiply charged ions and method 2 was mainly used for depleting the contaminants usually singly charged background ions, respectively. The setting parameters are described in Table 1.

TIMS funnel's voltages were linearly calibrated using Agilent ESI-L Tuning Mix to obtain reduced ion mobility coefficients ($1/K_0$) for three selected ions (m/z 622, 922, 1222) [7]. The $1/K_0$ was converted to CCS using the Mason-Schamp equation [8].

$$CCS = \frac{3ze}{16n_0}\sqrt{\frac{2\pi}{\mu k_B T}}\frac{1}{K_0} \qquad (1)$$

Table 1. Parameter settings of timsTOF Pro mass spectrometer in PASEF analysis.

Spray voltage	4000 V	
RF potential on electrodynamic funnel	350 Vpp	
Mode of IMS Separation	Method 1	Method 2
PASEF number	5	10
Potential for ramp start	180 V	130 v
Ramp time	250 ms	100 ms
Scan range of $1/K_0$	0.65 – 2.27 V·s/cm^2	0.7 – 1.40 V·s/cm^2
Scan range of m/z for MS and MS/MS	100 – 1750 m/z	100 – 1700 m/z
Target value for PASEF-MS/MS scan	2.40e + 04	
dynamic exclusion	25 s	
Step-up in collision energy		
Ramp time	collision energy	
0 –19%	52 eV	
19 –38%	47 eV	
38–57%	42 eV	
57–76%	37 eV	
76–100%	32 eV	
Isolation width of quadrupole (Th)		
m/z range	Charge dependent isolation width (1+/2+/3+)	
<200	4.0/3.0/3.0	2.0/2.0/2.0
200–700	5.0/4.0/4.0	2.0/2.0/2.0
700–800	5.0/4.0/4.0	3.0/3.0/3.0
800–1500	6.0/5.0/4.0	3.0/3.0/3.0
>1500	7.0/6.0/5.0	3.0/3.0/3.0

The z is the charge of the ions, e is the elemental charge (1.602×10^{-19} A·s), n_0 is Loschmidt constant (2.686×10^{25} m^{-3}), kB is Boltzman's constant (1.380×10^{-23} $kg \cdot m^2 \cdot K^{-1} \cdot s^{-2}$), μ is the reduced mass ($m_i m_g$ / (m_i + m_g), mi is the mass of ion; mg is the mass of N_2, 1 Da = 1.660×10^{-27} kg), K_0 is the reduced mobility, (10^{-4} $cm^2 \cdot V^{-1} \cdot s^{-1}$) and T is the temperature (305 K). For the CCS calculation, pure N_2 is assumed as the drift gas.

1.4. Data analysis

The peak list in mascot generic format (MGF) was generated by MaxQuant v1.6.7.0 [9], encoding information on both retention time and $1/K_0$ for each spectrum. The peptides were identified using X!Tandem Cyclone (12.10.01.1) [10] against human subset of the Swiss-Prot database (July 2016 extraction) or the *E. coli* K-12 MG1665 protein sequence database [11] with 20 ppm mass tolerance for both precursor and product ions. Carbamidomethyl (C) was set as a fixed modification. Oxidation (M, W), deamidation (N, Q), cyclization (N-term Q, C) and acetylation (protein N-term) were allowed as variable modifications, and strict enzymatic specificity allowing for up to 2 missed cleavages as search parameters. Redundant peptide identifications have been removed leaving the most intense peptide MS/MS hits with their correspondent $1/K_0$ and retention time values. Peptides with variable modifications were also removed for CCS prediction. All peptides with confidence score log(e) < -1 or better were additionally filtered using the latest version of SSRCalc retention time prediction model [12]. All peptides with retention time prediction error of more than ±6 min and low confidence score ($-3 <$ log(e) <-1) have been removed.

2. Data description

In the present work, 96 LC/MS/MS runs were conducted for 7 different enzymatic digestion samples with 7 SCX fractionations. As a result, 133,946 peptides of four different charge states were identified from the HeLa cells. For *E. coli*, 14,642 peptides of four different charge states were identified.

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Supporting Information

Supporting information is available online at https://dx.doi.org/10.14889/jpdm.2022.0003