RESOLUTE

Lab protocols to study Solute Carrier Transporters

Mass Spectrometry analysis (data acquisition)

ID: SP000S-Z

Authors	[1]André C. Mueller, [1]Eva Liñeiro
Affiliations	[1]CEMM - Research Center for Molecular Medicine. Vienna, AT.

The RESOLUTE project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 777372. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

Protocol description

Shotgun proteomics is a bottom-up approach use for the identification and quantification of proteins in complex mixtures by using high-performance liquid chromatography couple to high-resolution mass spectrometry. In a typical shotgun proteomics experiment, all proteins in the sample are enzymatically digested (usually by trypsin) to peptides. The obtained peptides are separate by liquid chromatography prior to be introduced in a mass spectrometer analyser. After MS analysis, the identification of peptides is reached by comparison of the mases of the peptides and their tandem mass spectra with the theoretical mases of proteolytic peptides generated in-silico from protein sequence databases. Protein inference is achieved by assigning identified peptide sequences to protein groups, and the measurement of mass spectral peaks intensities and/or spectral count are used for protein abundance quantification.

We use a shotgun proteomics approach to determine the SLC interactome landscape. Mass spectra were acquired by nano flow reverse-phase chromatography coupled to tandem mass spectrometry (LC-MS/MS). Mass spectra were acquired on a Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer (Thermo Fisher Scientific) in data-dependent acquisition (DDA) mode. Instrument performance was monitored by a quality control routine using BSA- and HeLa digest standards.

Materials

Biological materials:	▪ **RESOLUTE AP-MS/MS or BioID-MS/MS samples** (A detailed description of protocols use to prepare these samples can be found in the corresponding RESOLUTE protocol). ▪ **SH-quant : Isotopic labeled peptide :** [NH2}AADITS(L)YK[COOH] (L): Heavy Leu (13C15N) (+15) (Wepf et al., 2009)
Reagents:	▪ **Acetonitrile for HPLC LC-MS Grade** Product: 83640.32 Manufacturer: VWR ▪ **Water for HPLC LC-MS Grade** Product: 83645.320 Manufacturer: VWR ▪ **TFA Uvasol (trifluoroacetic acid)** Product: 1.08262.0100 Manufacturer: Merck ▪ **Methanol for HPLC LC-MS Grade** Product: 83638.32 Manufacturer: VWR ▪ **Suprapur ® Formic acid 98-100%** Product: 1.11670.1000

	Manufacturer: Merk ▪ **Pierce™ HeLa Protein Digest Standard** Product: 88328 Manufacturer: Thermo Fisher ▪ **Tryptic digested Bovine Serum Albumin** Product: 238031 Manufacturer: Roche ▪ **Pierce™ Quantitative Fluorometric Peptide Assay** Product: 23290 Manufacturer: Thermo Fisher Scientific ▪ **Pre-Diluted protein standard BSA** Product: 23208 Manufacturer: Thermo Fisher
Equipment:	▪ Nano-flow HPLC (Dionex Ultimate 3000 RSLC nano system (Thermo Scientific) ▪ Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer (Thermo Scientific) ▪ Nanospray Flex™ Ion Sources ES071 (Thermo Scientific) ▪ Column oven PRSOV2 (Sonation lab solutions)

Reagents setup

- **Nano pump solvent A: (Mobil non-polar/aqueous phase A):**
  - Composition: 0.4% FA in H_2O
  - For 100 mL: Mix 99.6 mL of H_2O with 400 µL of FA
  - Degas solvent by sonication (20 min in a sonicator bath). Replace MS-buffers every 2 weeks.

- **Nano pump solvent B (Mobil polar/organic phase B):**
  - Composition: 90% ACN, 0.4% FA in H_2O
  - For 100 mL: Mix 90 mL of ACN, 400 µL of FA and 9.6 mL of H_2O
  - Degas solvent by sonication (20 min in a sonicator bath). Replace MS-buffers every 2 weeks.

- **Loading pump solvent A (Mobil non-polar/aqueous phase A):**
  - Composition: 0.1% TFA in H_2O
  - For 500 mL: Mix 99.5 mL of H_2O and 500 µL of TFA
  - Degas solvent by sonication (20 min in a sonicator bath). Replace MS-buffers every 2 weeks.

- **Loading pump solvent B (Mobil polar/organic phase B):**
  - Composition: 70% MeOH, 0.1% FA in H_2O

- For 250 mL: Mix 175 mL of MeOH, 250 µL of TFA and 75 mL of H2O
- Degas solvent by sonication (20 min in a sonicator bath). Replace MS-buffers every 2 weeks.

- **30% TFA in water** (50 mL): Mix 15 mL of TFA with 35 mL of H_2O. Store at 4 ºC.

- **1% TFA in water** (50 mL): Mix 166 µl of 30% TFA solution with 49.8 mL of of H_2O. Store at 4 ºC.

Procedure

A. Peptide reconstitutions and quantification:

1. Peptide reconstitution
   - Before MS analysis reconstitute dry peptide samples in 32 µL of 0.1% TFA, with spiked in 16.66 fmol/µl SH-quant isotopic label peptide, mix by vortex.
   - Spin down samples and sonicate for 10 min in a sonicator bath.
   - Centrifuge (14.000 rpm., 4 °C, 15 min).
   - Transfer supernatant to a glass vial with insert.

2. Peptide quantification
   - Determine peptide concentration in samples by fluorometric peptide quantification assay according to manufactures protocol. Total peptide concentration should be similar from sample to sample, else dilute samples to standardize peptide concentration. Peptide concentration for MS-injections should be in between 250ng and 500ng.

B. Sample acquisition (nLC-MS/MS analysis)

- Mass spectrometry was performed on a Q Exactive™ hybrid quadrupole-Orbitrap mass spectrometer (ThermoFisher Scientific) coupled to an Dionex Ultimate 3000 RSLC nano system (ThermoFisher Scientific) via nanoflex source interface.

1. Peptide separation by nano-HPLC:
   - Peptides were separated by reverse-phase chromatography in a 50 cm, 75 µm inner diameter, analytical column (packed in house with ReproSil-Pur 120 C18-AQ, 3µm), using a nano-flow HPLC (Dionex Ultimate 3000, Thermo Fisher Scientific). Elution was achieved at a constant column oven temperature of 40 °C (Sonation column oven PRSO-V1. Sonation lab solutions). Peptides were loaded onto a trap column (Pepmap 100 5 µm, 5 × 0.3 mm, ThermoFisher Scientific) at a flow rate of 10 µL/min 0.1% TFA as loading buffer. After loading, the trap column was switched in-line with the analytical column. The flow rate was set to 230 nL/min and a 120 min gradient was used (4 to 24% solvent B within 24 min, 24 to 36% solvent B within 12 min and, 36 to 100% solvent B within 1 min, 100% solvent B for 6 min before equilibrating at 4% solvent B for 18 min).

2. Peptide ionization by ESI (Electro Spray Ionization):
    - Eluted peptides were ionized in a positive mode (1.8 Kv) using a Nanospray Flex™ Ion Source.

3. Tandem Mass spectrometry (MS/MS) analysis:
    - Analysis (MS/MS) was performed in a data-dependent acquisition mode. Full MS scans were acquired with a mass range of 375 - 1650 m/z in the orbitrap at a resolution of 70,000 (at 200 Da). Automatic gain control (AGC) was set to a target of 1×10^6 with a maximum injection time of 55 ms. Precursor ions for MS2 analysis were selected using a Top15 dependent scan approach using a quadrupole isolation window of 1.6 Da and higher energy collision induced dissociation (HCD) at a normalized collision energy (NCE) of 28%. AGC target was set to 1×10^5 with a maximum injection time of 110 ms and an Orbitrap resolution of 17,500 (at 200 Da). Dynamic exclusion for selected ions was 40 s. A single lock mass at m/z 445.120024 was employed for internal recalibration during the run.

Note: To ensure that chromatography and MS capabilities are stable and comparable across MS-injections, the performance of nLC-MS/MS needs to be continually monitored. For this purpose, it is advisable to acquire standard samples of tryptic digested Bovine Serum Albumin (BSA) before and after analytical sample and acquire HeLa Protein Digest Standard periodically.

Additional notes

- Section: Reagent set up:
    - Always use LC-MS grade water for the preparation of all the solvents.

Data availability

These settings were used for the MS analysis of all RESOLUTE samples. Data is accessible on our consortium website re-solute.eu under: https://re-solute.eu/resources/dashboards

References

- Wepf, A., Glatter, T., Schmidt, A., Aebersold, R. & Gstaiger, M. Quantitative interaction proteomics using mass spectrometry. Nat. Methods 6, 203–205 (2009).

Please write to contact@re-solute.eu in case of questions or errors.