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Data Article

Spiked human *substantia nigra* proteome data set for use as a spectral library for protein modelling and protein mappingSimone Steinbach^a, Bettina Serschnitzki^a, Manfred Gerlach^b,
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ABSTRACT

This article describes a mass spectrometric data set generated from human *substantia nigra* tissue that was spiked with iRT peptides. The data set can be used as a spectral library for analysis of the human brain; especially for analysis of human *substantia nigra*, for example, in the context of Parkinson's disease. Obtaining a sufficient amount of high-quality *substantia nigra* tissue is the key limiting factor for establishing a brain region-specific spectral library. Hence, combining existing spectral libraries for data-independent acquisition analysis (DIA) can overcome this major limitation. Moreover, these data can be used to map brain region-specific proteins and to model brain region-specific pathways. Both can improve our understanding of the functioning of the brain in greater depth. In addition, these data can also be used to determine the optimal settings for measuring proteins and peptides of interest. To create the *substantia nigra*-specific spectral library, the tissue was first homogenized and then fractionated via different types of SDS gel electrophoresis, resulting in 18 fractions. These fractions were analysed in triplicate by nanoHPLC-ESI-MS/MS, resulting in 54 data files. The data files generated from the

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described workflow are hosted in the public repository ProteomeXchange with the identifier PXD011076.

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Specifications Table

Subject area	Proteomics
More specific subject area	DIA-based mass spectrometric analysis of human brain tissue
Type of data	Raw files, msf files
How data was acquired	Mass spectrometry (Q Exactive HF mass spectrometer operated in data-dependent acquisition (DDA) mode performing HCD fragmentation)
Data format	Raw, unfiltered
Experimental factors	Data were obtained by mass spectrometric DDA measurements of human substantia nigra tissue spiked with iRT peptides for generation of a spectral library
Experimental features	Substantia nigra tissue was fractionated via gel electrophoresis and in-gel digested, resulting in 18 fractions. Bands were measured in triplicate, resulting in 54 files
Data source location	Bochum, Germany (51°26'43.4"N 7°15'27.9"E)
Data accessibility	The data files are hosted in the public repository ProteomeXchange with identifier PXD011076.
Related research article	Barkovits K., Linden A., Galozzi S., Schilde L., Pacharra S., Mollenhauer B., Stoepel N., Steinbach S., May C., Uszkoreit J., Eisenacher M., Marucs K.: Characterization of Cerebrospinal Fluid via Data-Independent Acquisition Mass Spectrometry [1] Published in J Proteome Res. 2018

Value of the data

The provided data set represents a standard proteomic data set of human *substantia nigra* tissue measured in DDA mode. The samples were spiked with iRT peptides.

- The spiked data set can be used as a spectral library for human brain tissue analysis in DIA mode, for example, in the context of Parkinson's disease.
- The library can be used to map proteins in different brain regions.
- The library may help to determine optimized parameters for the identification of proteins/peptides of interest.
- The library can be used to model pathways located within specific brain regions.

1. Data

The proteomic data set provided here includes mass spectrometric data generated from human *substantia nigra* tissue. The data set was generated from human *substantia nigra* tissue, that was lysed and fractionated by one-dimensional SDS polyacrylamide gel electrophoresis (see Fig. 1). The resulting lanes were cut into single bands and proteins were in-gel digested with trypsin. After peptide extraction the samples were spiked with iRT peptides and measured with a data-dependent mass spectrometric approach (DDA). The data set from human *substantia nigra* here provided is the most extensive and most advanced one regarding separation strategy and mass spectrometric analysis, compared to previous analyses, as e.g. from Licker et al. [2,3].

2. Experimental design, materials and methods

For a detailed overview of the workflow see Fig. 1.

2.1. Sample preparation

Substantia nigra tissue was dissected as described by Plum et al. [4]. For protein extraction, *substantia nigra* was first dissected into smaller pieces at 4 °C and transferred to 1.5 mL reaction tubes. Per

100 mg of tissue, 400 μ L of precooled RIPA buffer (Cell Signalling, Germany) was added along with 0.35 g of precooled glass beads (\emptyset 1.25–1.65 nm; \emptyset 0.25–0.5 nm; Carl Roth, Germany). The samples were homogenized by sonication with an ultrasound probe (Potter S. Homogenizer, B. Braun, Germany) precooled at 4 °C with an amplitude of 90 and a power of 50% for 1 min for a total of four times. The supernatants were transferred to new 1.5 mL reaction tubes. The remaining glass beads in each tube were washed by adding 50 μ L of precooled ultrapure water and vortexing, and the supernatants were merged with the samples transferred previously. The remaining reaction tubes and the glass beads were discarded, and the merged samples were again sonicated in an ice-cold ultrasonic bath (BANDELIN electronic GmbH & Co. KG, Germany) for 10 sec for a total of six times. To remove the insoluble components, the samples were centrifuged at 16.000 g for 15 min at 4 °C (centrifuge 5415R, Eppendorf GmbH, Germany). All supernatants were merged in a 5 mL reaction tube and vortexed. All remaining reaction tubes, along with the sediments, were discarded. To remove the remaining lipids, the merged sample underwent a further clean-up step involving acetone precipitation. For this, the tissue lysate was solved 1:4 (v/v) in acetone (J.T. Baker, the Netherlands) and incubated at –20 °C overnight. To remove the acetone from the precipitated protein, the sample was again centrifuged for 4 min at 5.000 g and 4 °C (centrifuge 5415R, Eppendorf GmbH). The supernatant was discarded, and the sediment was air dried in the opened reaction tube for 5 min on ice. Finally, the sediment was solubilized by adding LDS sample buffer (pH 8.5), which contained 26.5 mM Tris HCl, 35.25 mM Tris base, 2% LDS, 10% glycerol, 0.055 mM Coomassie blue G250 and 0.045 mM phenol red, and vortexing. The tissue lysate was subsequently aliquoted and stored at –80 °C. The protein concentration of the tissue lysate was determined with a Bradford assay (Sigma-Aldrich, Germany) that was operated according to the manufacturer's instructions. A total of 500 μ g of the tissue lysate was transferred to a 0.5 mL reaction tube. For reduction of the disulphide bridges and denaturation, 1:10 (v/v) 2 mM DTT was added, followed by incubation for 10 min at 350 rpm and 90 °C in a thermomixer (Thermomixer comfort, Eppendorf GmbH). For removal of the insoluble components and condensation of the buffer in the lid of the reaction tube, the sample was again centrifuged for 4 min at 5.000 g at room temperature (centrifuge 5415R, Eppendorf GmbH).

2.2. Complete SDS gel electrophoresis

For this, 40 μ g of protein per lane was loaded on a NuPAGE™ 4–12% Bis-tris gel or on a NuPAGE™ 3–8% Tris-acetate gel (both from Fisher Scientific, Germany). The different types of gel were chosen to cover a large molecular weight range. The running buffers were prepared, and gel electrophoresis was performed according to the manufacturer's instructions.

2.3. Gel staining

Next, the gels were stained with Coomassie blue (SimpleBlue™ SafeStain, Thermo Fisher Scientific) according to the manufacturer's protocol.

2.4. Trypsin in-gel digestion

Lanes on the NuPAGE™ 4–12% Bis-tris gel were cut into 13 single bands, and lanes on the NuPAGE™ 3–8% Tris-acetate gel into 10 single bands (of which 5 were used for further analysis, because they separated proteins with a molecular weight, which was not covered by the NuPAGE™ 4–12% Bis-tris gel). Summing up the lanes of both gels lead to 18 bands. Each band was transferred into a glass vial, destained and pH adjusted by incubating the gel pieces first for 10 min in 50 mM ammonium bicarbonate (Sigma-Aldrich) and afterwards in 50% (v/v) 50 mM ammonium bicarbonate with 50% (v/v) 100% acetonitrile (Merck KGaA, Germany). The incubation cycle was repeated three times in total. For reduction and modification of the disulphide bridges after the second incubation with 50 mM ammonium bicarbonate, the ammonium bicarbonate buffer was discarded, and each glass vial was filled with 50 μ L of 10 mM dithiothreitol (AppliChem GmbH, Germany), followed by incubation for 1 hour at 350 rpm and 56 °C in a thermomixer (Thermomixer comfort, Eppendorf GmbH). Then, the dithiothreitol solution was again discarded, and each glass vial was filled with 50 μ L of 50 mM

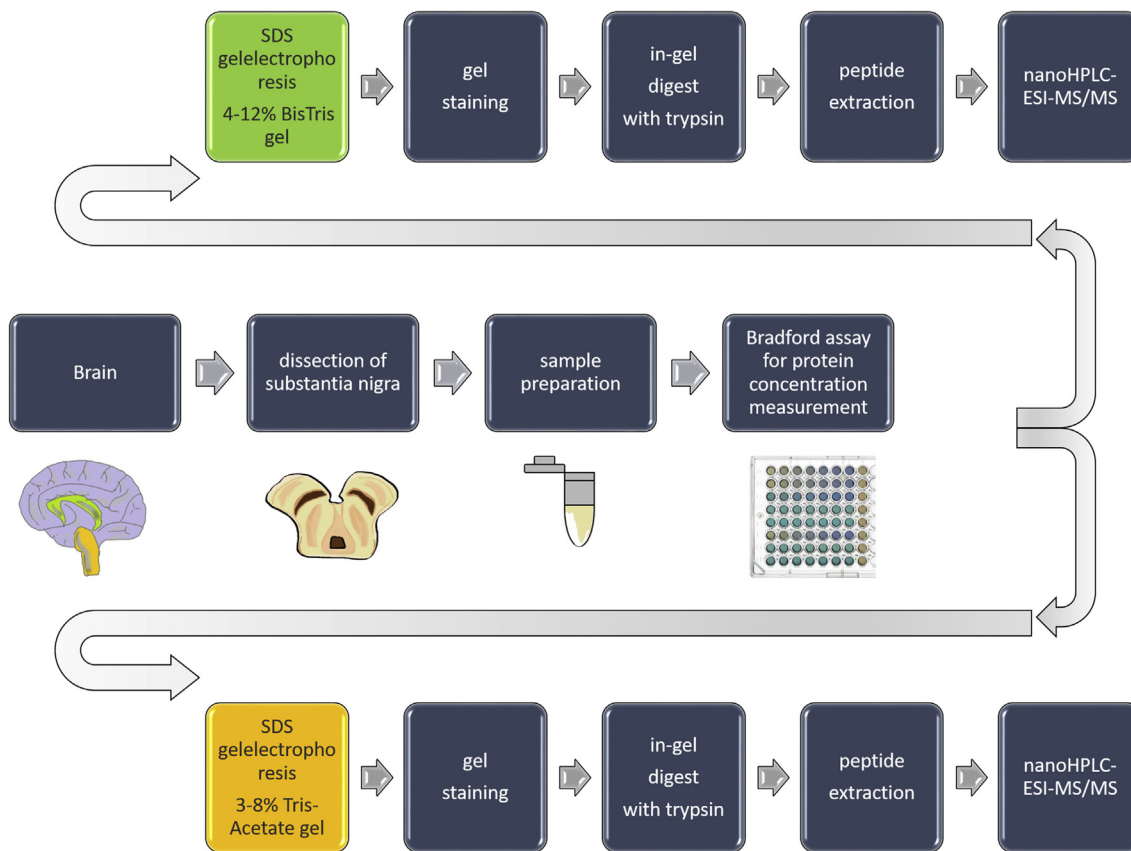


Fig. 1. Graphical workflow overview. *Substantia nigra* tissue was first homogenized, and the protein concentration in the resulting lysate was determined. For fractionation of the *substantia nigra* lysate, the lysate was completely fractionated (18 fractions) using two types of gradient SDS gels (3–8% Tris-acetate and 4–12% Bis-tris) to cover a width range of molecular weights. In total, 54 samples (3 replicates of each fraction) were generated for nanoHPLC-ESI-MS/MS analysis.

iodoacetamide (Merck KGaA) followed by incubation for 45 min at room temperature in the dark. Subsequently, the iodoacetamide solution was discarded, and the destaining protocol was continued with 50% (v/v) 50 mM ammonium bicarbonate with 50% (v/v) 100% acetonitrile.

2.5. Peptide extraction

The gel pieces were dried in a vacuum concentrator (RVC2-25CD plus, Martin Christ Gefrier-trocknungsanlagen, Germany) and resuspended in 6 μ L of trypsin solution (0.012 μ g/ μ L, Promega Corp., Germany). Digestion was performed overnight. The digestion was stopped, and peptides were eluted by incubating the gel pieces twice for 15 min in 30 μ L of a 1:1 solution containing 100% acetonitrile and 0.1% (v/v) trifluoroacetic acid (Merck KGaA) in an ice-cold ultrasonic bath. All of the peptide extract resulting from the bands of the complete SDS gel electrophoresis was used for nanoHPLC-ESI-MS/MS because the peptide concentration is, according to our experience, no higher than 500 ng per band and therefore does not exceed the capacity of the nanoHPLC. For the samples resulting from the whole *substantia nigra* lysates, 800 ng of each peptide extract was used for nanoHPLC-ESI-MS/MS. The peptide concentrations of these samples were determined by amino acid analysis.

2.6. Amino acid analysis

The glass vials for the acidic gas-phase hydrolysis were first heated at 400 $^{\circ}$ C for 4 hours (muffle furnace, Carbolite CWF 1100, USA) to remove any contamination. From each sample, duplicate determinations were made. For acidic hydrolysis, 6 μ L of each peptide extract (in the following referred to as samples) was transferred to a new glass vial, dried in a vacuum concentrator (RVC2-25CD plus) and then placed in an evacuation vessel. Afterwards, 400 μ L of 6 M HCl and a phenol crystal were added to the samples, followed by alternating evacuation and aeration with argon four times. The hydrolysis was performed for 1 hour at 150 $^{\circ}$ C. To remove residual HCl, the peptide samples were evacuated again. For derivatization, 30 μ L of AccQ-Fluor borate buffer with the internal standard Norleucine and 10 μ L of AccQ-Fluor reagent (10 mM 6-aminoquinolyl-*N*-hydroxysuccinimidylcarbamate in acetonitrile) were added to the hydrolysed samples and incubated for 10 min at 55 $^{\circ}$ C. Primary and secondary amines were converted to stable derivatives. The derivatives were separated on a C18 reversed-phase separation column (2.1 mm \times 100 mm in length, Waters GmbH, Germany). After derivatization with the derivatization reagent AccQ-Fluor, the hydrolysed peptides were dissolved in 10 μ L of 20 mM HCl. A gradient system consisting of two solvents was used for the separation (solvent A: AccQ-Tag Ultra Eluent A; solvent B: AccQ-Tag Ultra Eluent B). Elution of the derivatives was performed at a flow rate of 0.7 mL/min and a column temperature of 55 $^{\circ}$ C by increasing the solvent A content in the solvent mixture (see Table 1). The amino acid derivatives were detected at an emission wavelength of 260 nm using a UV detector (Waters GmbH). Quantitative analyses were performed using different concentrations of an internal amino acid standard.

2.7. Peptide spiking

The samples were transferred to glass vials, dried in a vacuum concentrator (Eppendorf GmbH) and resuspended in 15 μ L of 0.1% (v/v) trifluoroacetic acid. Finally, the samples were spiked with one injection volume of iRT peptides (Biognosys, Switzerland) according to manufacturer's instructions and then analysed via nanoHPLC-ESI-MS/MS.

2.8. nanoHPLC settings

Prior to mass spectrometric analysis, the extracted peptides were first concentrated and cleaned-up using a nanoHPLC system (UltiMate 3000, Dionex, Germany) with an integrated capillary pre-column (100 μ m \times 2 cm, particle size 5 μ m, pore size 100 \AA ; Thermo Scientific, USA), which was washed for 7 min with 0.1% trifluoroacetic acid. Then, the peptides were eluted from the pre-column to an analytical column (PepMapTM C18 75 μ m \times 50 cm, particle size 2 μ m, pore size 100 \AA ; Thermo Fisher Scientific) before peptide separation was performed by a 1 min long segmented gradient of solvent A (0.1% formic

Table 1
Solvent gradient profile for the elution of amino acids.

Time (min)	% Solvent A	% Solvent B
0	99.9	0.1
0.54	99.9	0.1
5.74	90.9	0.1
7.74	78.8	21.2
8.04	40.4	59.6
8.05	10.0	90
8.64	10.0	90
8.73	99.9	0.1
9.50	99.9	0.1

acid) and solvent B (84% acetonitrile, 0.1% formic acid) (see Table 2). The concentration of solvent B was increased from 5% to 60%. The column oven temperature was set to 60 °C, and the flow rate was 400 nL/min. The nanoHPLC system was directly coupled to a nano electrospray ionization source (Thermo Fisher Scientific).

2.9. Mass spectrometry settings

Subsequently, the peptides were ionized by electrospray ionization and injected into a Q Exactive™ HF mass spectrometer (Thermo Fisher Scientific, Germany) operating in data-dependent acquisition mode. The mass spectra were recorded between 350 and 1100 m/z with a resolution of 120,000 (AGC 3e6, 80 ms maximum injection time, 2.2 m/z wide isolation window). The capillary temperature was set to 250 °C, and the spray voltage was set to 1600 V. For internal recalibration, the lock mass of polydimethylcyclosiloxane (445.120 m/z) was used. HCD fragmentation of the top-10 abundant precursor ions at 27% NCE was performed, followed by fragment ion analysis with an orbitrap mass analyser with a resolution of 30,000 at 200 m/z (AGC 5e5, 120 ms maximum injection time). LC-MS/MS analysis of the *substantia nigra* fractions was performed with slight modifications. The HPLC gradient was a 141 min segmented gradient from 5 to 60% (v/v) solvent B. The mass range of the mass spectrometric analysis was set to 350–1100 m/z with a resolution of 120,000.

Table 2
Solvent gradient profile for the elution of peptides.

Time (min)	% Solvent B
0	5
7	5
20	8
30	10
45	12
75	15
105	19
113	21
118	23
121	25
124	27
127	30
129	34
132	37
136	43
142	52
148	60
149	95
154	95
155	5
160	5

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Transparency document

Transparency document associated with this article can be found in the online version at <https://doi.org/10.1016/j.dib.2019.103711>.

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