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Data Article

# Rat retinae data for use as spectral library, for pathway remodeling as well as protein mapping

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ABSTRACT

This article describes a mass spectrometric data set from rat retinae spiked with indexed Retention Time (iRT) peptides. The provided data set can be used as a spectral library to investigate for instance eye disorders as well as ocular function by data-independent acquisition (DIA) based mass spectrometry. Consequently, there is no urgent need to create an own spectral library, which requires money, time, effort as well as tissue. Besides the use as a spectral library, this data set can improve our knowledge about proteins present in the rat retina and thus the protein pathways within this tissue. The data set may also help to determine optimal parameters for peptide identification by mass spectrometry. To generate the presented data set, six rat retinae were homogenized with glass beads and pooled. The pooled sample was fractionated by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) followed by tryptic in-gel digestion. The fractionation of the pooled sample was repeated for further 4 times, to end up with in total 5 technical replicates. Peptide extracts were spiked with iRT peptides and analyzed

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by data-dependent (DDA) nanoHPLC-ESI-MS/MS resulting in 60 files. All resulting data files are hosted in the public repository ProteomeXchange under the identifier PXD021937.



## Specifications Table

| | |
|---|---|
| Subject | Proteomics |
| Specific subject area | DIA based mass spectrometric analysis of rat retinae |
| Type of data | Mass spectrometric raw data (DDA) |
| How data were acquired | Mass spectrometry (Q Exactive mass spectrometer operated in DDA mode performing HCD fragmentation) |
| Data format | RAW, mzXML data,<br>unfiltered |
| Parameters for data collection | Intensity, retention time and charge of fragmented tryptic peptides were obtained by mass spectrometric DDA measurements of rat retinae. |
| Description of data collection | The rat retina samples were prepared, pooled and fractionated by gel electrophoresis resulting in 12 bands and 4 additional technical replicates. All samples were digested with trypsin. The samples were measured using nanoHPLC-MS/MS in DDA mode. |
| Data source location | Ruhr-University Bochum, Center for Protein Diagnostics (PRODI), Medical Proteome Analysis, Bochum, Germany (51°26′43.4″N 7°15′27.9″E) |
| Data accessibility | Repository name: ProteomeXchange<br>Data identification number: PXD021937<br>Direct URL to data: http://www.proteomexchange.org/<br>Excel file of analyzed data in the supplement |
| Related research article | [3] Noristani, R.; Kuehn, S.; Stute, G.; et al. (2016): Retinal and Optic Nerve Damage is Associated with Early Glial Responses in an Experimental Autoimmune Glaucoma Model. J Mol Neurosci 58, 4, 470-482. http://dx.doi.org/10.1007/s12031-015-0707-2.<br>[4] Reinehr, S.; Reinhard, J.; Gandej, M.; et al. (2018): S100B immunization triggers NFκB and complement activation in an autoimmune glaucoma model. Sci Rep 8, 1, 9821. http://dx.doi.org/10.1038/s41598-018-28183-6.<br>Benning L, Reinehr S, Grotegut P, Kuehn S, Stute G, Dick HB, Joachim SC. Synapse and Receptor Alterations in Two Different S100B-Induced Glaucoma-Like Models. Int J Mol Sci. 2020 Sep 23;21(19):6998. http://dx.doi.org/10.3390/ijms21196998. |

## Value of the Data

- The data set can be used as a rat retinae specific spectral library for research projects that focus on eye function or disorders.
- It may help to expand the knowledge of the retina proteome.
- It may help to uncover protein pathways within the retina.
- It may help optimizing parameters for mass spectrometric identification of peptides as well as proteins.

## 1. Data Description

The dataset presented here was generated by nanoHPLC-MS/MS analysis of protein extracts from six rat retinae. Retinae were prepared from rats followed by protein extraction using glass beads. Protein extracts were pooled, and the subsequent analysis performed with a total of five technical replicates. Proteins were separated by SDS-PAGE, visualized with Coomassie Brilliant

Blue and gel lanes were cut into single equal size pieces. Proteins were tryptically digested and resulting peptides were eluted from the gel. Indexed Retention Time (iRT) peptides were added to each sample. All samples were measured in DDA mode. All raw data (RAW and mzXML-format) are provided in PRIDE. The analyzed data are provided as Excel file in the supplement

## 2. Experimental Design, Materials and Methods

### *2.1. Retina preparation*

Male Brown-Norway rats (8 to 10 weeks old, Charles River Laboratories) were scarified by carbon dioxide inhalation (n=6). The left eye of each animal was enucleated and transferred into a PBS filled petri dish. Next, the eyeball was opened with a scissor to separate anterior parts of the eye from the eye cup. Afterwards, each retina was carefully extracted from the eye cup, transferred in a 1.5 mL reaction tube, snap frozen in liquid nitrogen, and stored at −80°C. Protein extraction was performed using glass beads as described previously by Steinbach et al. [1]. Precooled 40 µL RIPA buffer (Cell Signaling) together with 0.035 g precooled glass beads (Ø 1.25-1.65 nm; Ø 0.25-0.5 nm; Carl Roth) was added to 10 mg tissue. Homogenization was performed by sonication using an ultrasound probe (Potter S. Homogenizer, B. Braun) precooled at 4°C for 1 min for in total four times. The amplitude of the ultrasound probe was set to 90 and the power to 50%. Resulting supernatants were transferred to a new 1.5 mL reaction tube and remaining glass beads washed with 5 µL precooled water. The resulting sample was merged with sample generated before and sonicated in an ice-cold ultrasonic bath (BANDELIN electronic GmbH Co.KG) for 10 sec for in total six times. For removal of possible insoluble components, samples were centrifuged at 16,000 g for 15 min at 4°C (centrifuge 5415R, Eppendorf GmbH), the supernatant transferred to a new reaction tube and the resulting sediments discarded.

### *2.2. Peptide concentration measurement using amino acid analysis*

Protein concentration determination for each of in total 5 retina lysates was performed by amino acid analysis as described by Guntermann et al. [2]. Briefly, to eliminate possible contaminations glass vials were incubated in a muffle furnace (muffle furnace, Carbolite CWF 1100) for 4 h at 400°C. Subsequently, 4 µL of each protein extract were transferred into a muffled glass vial and dried using a vacuum concentrator (RVC2-25CD plus, Martin Christ Gefriertrocknungsanlagen). Afterwards, glass vials were placed in an evacuation vessel together with 400 µL 6 M hydrochloric acid as well as a phenol crystal. All samples were alternately evacuated for a total of four times and aerated with argon. Using acidic gas phase hydrolysis, proteins were hydrolyzed into single amino acids. In a further evacuation step, remaining hydrochloric acid was removed. For derivatization, 10 µL of 20 mM hydrochloric acid, 30 µL AccQ fluoroborate buffer with the internal standard Norvaline as well as 10 µL AccQ fluor reagent (10 mM 6-aminoquinolyl-N-hydroxysuccinimidylcarbamate in acetonitrile) were added. Hereafter, samples were incubated for 10 min at 55°C and the resulting derivatives separated on a C18 reversed-phase separation column (2.1 mm × 100 mm length, Waters GmbH). A gradient based on two solvents (solvent A: AccQ-Tag Ultra Eluent A, solvent B: AccQ-Tag Ultra Eluent B) was used for elution, which was performed with a flow rate of 0.7 mL/min and a column temperature of 55°C. In order to detect amino acid derivates, an UV-detector (Waters GmbH) was utilized for the detection of amino acid derivatives. For quantification, different concentrations of an internal amino acid standard were compared with the original sample. The sum of all calculated amino acid concentrations was taken as total protein concentration within the sample. Of each retina lysate, 20 µg protein were used to prepare a pooled sample for further analysis.

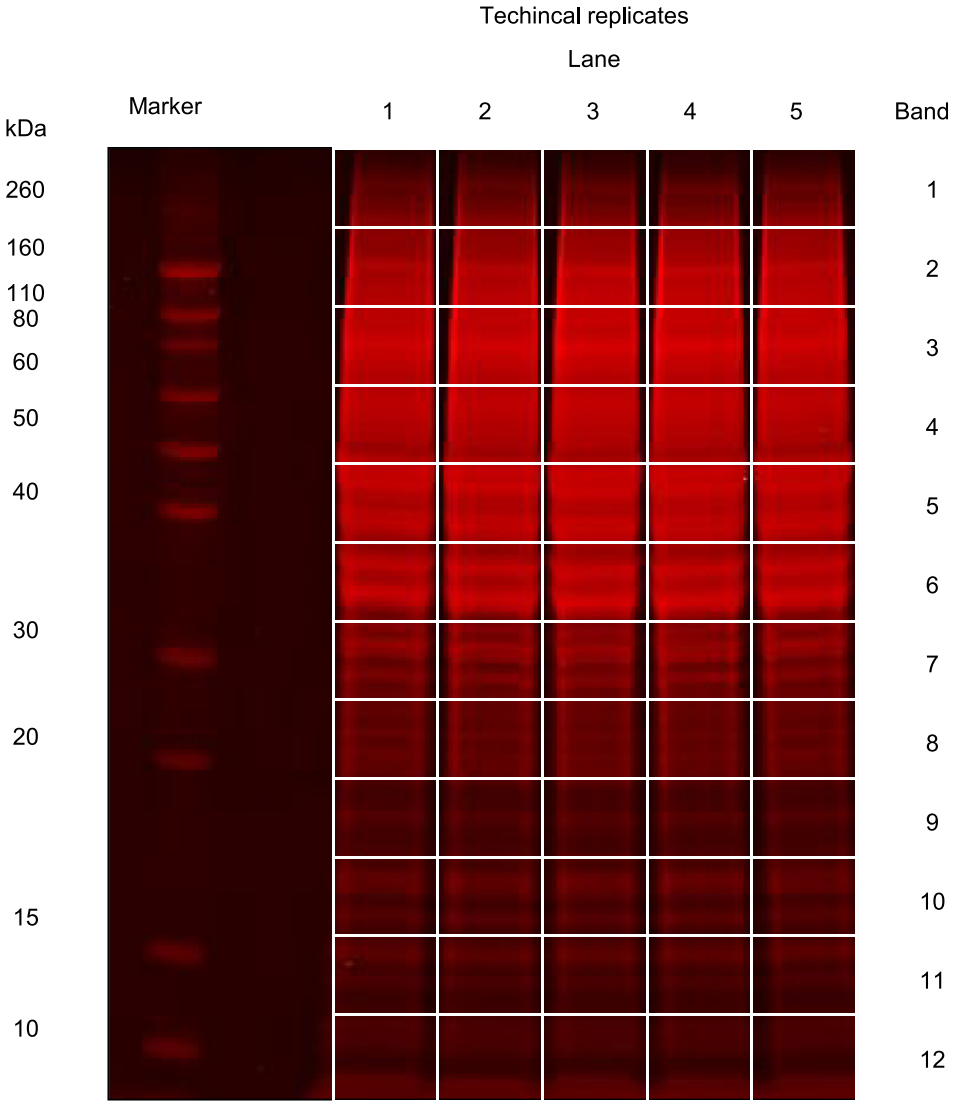


**Fig. 1. Coomassie Brilliant Blue stained SDS gel of retina lysates.** Pooled retina lysate was separated in replicates by SDSPAGE. After electrophoresis, the gel was stained with Coomassie Brilliant Blue and lanes were cut in equal size gel pieces. Gel pieces underwent in-gel tryptic digest and finally analyzed by mass spectrometry.

### *2.3. Gel electrophoresis*

In total 80 µg retinal protein extract was loaded per lane on a NuPAGE$^{TM}$ 4-12% Bis-Tris gel (Thermo Fisher Scientific Corp.). To obtain technical replicates, this was repeated for 5 times (Fig. 1). In detail, to 80 µg of lysate 4xLDS sample buffer was added (pH 8.5) containing 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. For reduction, additional reducing agent (Thermo Fisher Scientific Corp.) according to manufacturer's instructions was added, samples mixed and incubated for 10 min at 90°C at 350 rpm in a thermomixer (Thermomixer comfort, Eppendorf GmbH). Condensed buffer

in the lid was removed by a short centrifugation step at room temperature for 4 min at 5,000 g (centrifuge 5415R, Eppendorf GmbH) and samples directly loaded onto the gel. SDS-PAGE was performed with the following voltage profile: 50 V for 15 min and 180 V for 50 min. After electrophoresis, the gel was stained with Coomassie Brilliant Blue (SimpleBlue™ SafeStain, Thermo Fisher Scientific Corp.) according to the manufacturer's instructions. Subsequently, the gel was scanned with the Odyssee® imaging system (LI-COR Biotechnology GmbH) in the 800 nm channel. The resulting image is displayed in Fig. 1 in red pseudo-color.

### 2.4. Destaining and in-gel digestion

Destaining and in-gel digestion were performed as described by Steinbach et al. [1]. Protein lanes were cut into 12 equal gel pieces and destained. For illustration purposes, the cutting areas are marked with a white grid (Fig. 1). Next, pH was adjusted by incubating the gel pieces alternately 3 times each for 10 min with 50% (v/v) 50 mM ammonium bicarbonate (Sigma-Aldrich) and with a mixture of 50% (v/v) 50 mM ammonium bicarbonate plus 100% acetonitrile (Merck KGaA). After the first incubation cycle, samples were reduced with 50 µL 10 mM 1,4-dithiothreitol (DTT; AppliChem GmbH) at 56°C for 1 h followed by alkylation with 50 µL 55 mM iodoacetamide (Merck KGaA) at room temperature in the dark for 45 min. Finally, gel pieces were dried in a vacuum concentrator (RVC2-25CD plus, Martin Christ Gefriertrocknungsanlagen) and resuspended in 6 µL 50 mM ammonium bicarbonate. Overnight digestion was initiated by adding 6 µL of trypsin solution (0.033 µg/µL, Promega Corp.). The digestion was stopped, and peptides eluted by incubating the gel pieces twice with 20 µL of a 1:1 solution containing 100% acetonitrile and 0.1% (v/v) trifluoroacetic acid (TFA) (Merck KGaA) in an ice-cooled ultrasonic bath for 15 min. Samples were transferred into glass vials, dried in a vacuum concentrator and resuspended in 20 µL 0.1% (v/v) TFA. The protein concentration was measured by amino acid analysis using a double determination of 4 µL [2]. At the end, samples were injected with 1 µL of iRT peptides (Biognosys AG).

### 2.5. High-performance liquid chromatography

Peptides were analyzed using high-performance liquid chromatography as described by Guntermann et al. [2]. First, extracted peptides were cleaned-up as well as concentrated using an UltiMate™ 3000 nanoHPLC system (Dionex, Thermo Fisher Scientific Corp.) containing an integrated capillary pre-column (100 µm × 2 cm, particle size 5 µm, pore size 100 Å; Thermo Scientific Corp.) and an analytical column (PepMap C18 75 µm × 50 cm, particle size 2 µm, pore size 100 Å; Thermo Scientific Corp.). Washing of the pre-column was performed with 0.1% TFA for 7 min. Afterwards, peptides were eluted from the pre-column onto the analytical column and separated by applying a segmented 160 min gradient of buffer A (0.1% fluoric acid) and buffer B (84% acetonitrile, 0.1% fluoric acid) with a flow rate of 400 nL $min^{-1}$ (Fig. 2 and Table 1). The column oven temperature was set to 60°C.

### 2.6. Mass spectrometry settings

Peptides were ionized by electrospray ionization (ESI), adjusting the capillary temperature to 250°C as well as the spray voltage to 1,600 V, followed by injection of the peptides into a Q Exactive™ mass spectrometer (Thermo Fisher Scientific Corp.). The instrument operated in DDA mode. Thus, it performed HCD fragmentation of the top 10 abundant precursor ions. For full scan mode, the scan range was defined as 350 – 1,100 m/z with a resolution of 120,000. Internal recalibration used a target (AGC) of 3e6 and a fill time of 20 ms. Fragment ion analysis was performed with the Orbitrap mass analyzer in a 1.6 m/z wide isolation window with a

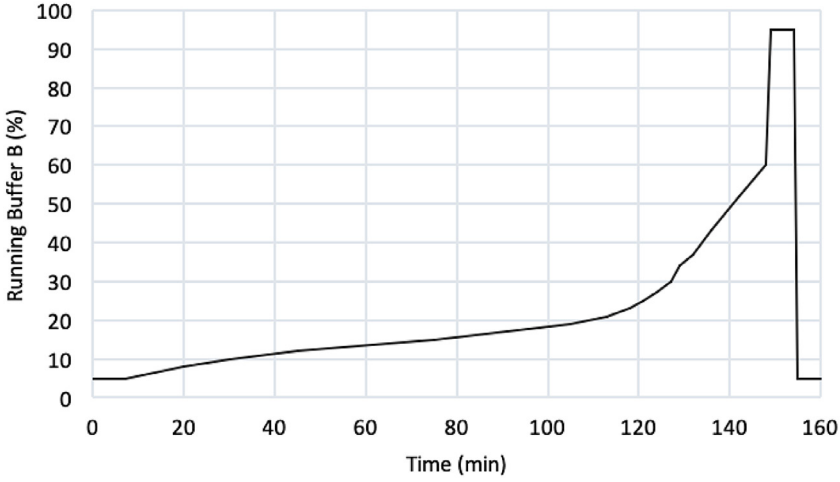


**Fig. 2. Solvent gradient profile for elution of peptides.** Peptide separation was performed by applying a segmented 160 min gradient. The gradient was run from 5 to 60% running buffer B for 148 min followed by a 5 min washing step at 95% as well as a 5 min equilibration step at 5% running buffer B.

**Table 1**
HPLC running buffer gradient profile.

| Time (min) | Running buffer 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 |

resolution of 30,000, whereby the fixed first mass was set to 100 m/z (AGC 1e5, maximum injection time: 25 ms, loop count: 10). Dynamic exclusion was applied within 20 s to limit the redundancy in selecting the same precursor ion over and over again. In-source CID collision energy was turned off (0.0 eV). Here, higher energy collision induced dissociation (HCD) at a normalized collision energy (NCE) was used as center energy. Q Exactive HF mass spectrometer performed a stepwise fragmentation on the precursor ion at a stepped NCE of 24%, 27% and 30% of this center energy. Note, the dimensionless unit of NCE is approximately equivalent to the HCD collision energy (in eV) for a reference ion of mass 500 and charge 1.

The resulting RAW-files were converted into the mzXML-format and both file formats uploaded to the public repository PRIDE ProteomeXchange under the identifier PXD021937. The spectra library was created using the Spectronaut$^{TM}$ Pulsar software (Biognosys AG). This involved matching the generated spectra of the DDA measurements with the theoretical spectra contained in the Uniprot/SwissProt rat proteome reference database (UniProtKB/Swiss-Prot UniProt release 2012_12; downloaded: 2020–12–16; number of entries: 8114). A total of 60 DDA measurements (12 fractions and 5 technical replicates of pooled samples) were utilized. For the generation of the spectral library, default settings were applied. False discovery rate, called "Qvalue", was set to a threshold of $\leq 0.01$. Finally, export of this spectral library was conducted in Excel format (Supplementary table 1). Here, the data set sheets are named with the corresponding gel lane and band number (Fig. 1) as well as the respective RAW-file number of the mass spectrometric analysis.

## Ethics Statement

All procedures concerning animals adhered to the ARVO statement for the use of animals in ophthalmic and vision research. All experiments involving animals were approved by the animal care committee of North Rhine-Westphalia, Germany (84-02.04.2016.A141), and were performed in accordance with relevant guidelines and regulations.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships, which have, or could be perceived to have, influenced the work reported in this article.

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## Supplementary Materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.dib.2021.107212.

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