Data Article

Labeled quantitative proteomics dataset of optogenetics induced axon regeneration in mice

Faith Christine Harvey\textsuperscript{a,b,c}, Ximena Mendoza\textsuperscript{b,d}, Yuan Liu\textsuperscript{a,b,c}, Richard K. Lee\textsuperscript{a,b,c,*}, Sanjoy K. Bhattacharya\textsuperscript{a,b,c,*}

\textsuperscript{a} Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL 33136, USA
\textsuperscript{b} Miami Integrative Metabolomics Research Center, Miami, FL 33136, USA
\textsuperscript{c} University of Miami Miller School of Medicine, Miami, FL 33136, USA
\textsuperscript{d} University of Miami, Miami, FL 33146, USA

A R T I C L E   I N F O

Article history:
Received 21 April 2022
Revised 12 May 2022
Accepted 17 May 2022
Available online 21 May 2022

Dataset link: Labeled quantitative proteomics dataset of optogenetics induced axon regeneration (Accession no. PXD32788) (Original data)

Keywords:
Axon regeneration
Optogenetic stimulation
Quantitative proteome
TMT-labeling

A B S T R A C T

This labeled quantitative proteomics dataset was collected from a transgenic channel rhodopsin mouse model (Chr2) subjected to light stimulation after traumatic optic nerve crush (ONC). Protein extraction was performed by careful mincing of the tissue in extraction buffer (TEAB, NaCl and SDS). Protein amounts were normalized across samples using dot blot densitometry and ImageJ software. Samples were labeled for quantification using a modified TMTpro\textsuperscript{TM} 16plex Label Reagent Set (Thermo Scientific\textsuperscript{TM}) after performing an overnight trypsin digestion. Untargeted liquid chromatography-mass spectrometry was performed on an Easy-nLC 1000 liquid chromatograph coupled to a Q Exactive mass spectrometer (LC-MS/MS). Data analysis was performed using Proteome Discoverer\textsuperscript{TM} 2.5 (Thermo Scientific\textsuperscript{TM}). This data has been deposited to the ProteomeXchange (PX) and is available through PRIDE with the identifier PXD032788.
Specifications Table

| Subject          | Ophthalmology                                      |
|------------------|----------------------------------------------------|
| Specific subject area | Quantitative proteomics in axon regeneration         |
| Type of data     | Table                                              |
|                   | Graph                                              |
|                   | Chromatogram                                       |
|                   | Figure                                             |
| How the data were acquired | LC-MS/MS                                       |
| Data format       | Raw, analyzed, and filtered                        |
| Description of data collection | A total of 36 optic nerves were collected from channelrhodopsin (Thy1-Chr2-eYFP) and control (C57BL/6J) mice at 1 and 2 weeks post-optic nerve crush. After protein extraction, digestion and TMT labeling, samples were analyzed with untargeted LC-MS/MS. |
| Data source location | Bascom Palmer Eye Institute, Miller School of Medicine at University of Miami, Miami, FL 33136, USA |
| Data accessibility | [ProteomeXchange identifier: PXD032788](https://www.ebi.ac.uk/pride/archive/login) |
|                   | Reviewer Account Details:                          |
|                   | Username: reviewer_pxd032788@ebi.ac.uk              |
|                   | Password: QjbjYJl                                   |

Value of the Data

- This data provides quantitative data of the protein changes after traumatic optic nerve crush and subsequent light stimulation promoted regeneration in Thy1-Chr2-eYFP mice and C57BL/6J control mice.
- This data can be used in the development of therapeutic treatments centered on the proteomic changes following traumatic optic neuropathies.
- This data can aid in the development of a comprehensive proteomic library that can be accessed for further research.

1. Data Description

The data presented here was generated from a quantitative mass spectrometry-based analysis studying the effects of optogenetic stimulation following optic nerve crush injury. Transgenic mice expressing the exogenous gene channelrhodopsin (Chr2) were studied for changes in the proteomic profile of the axons following optic nerve crush. While a lipidomics dataset is already available for this regeneration model, this is the first quantitative proteomics dataset for optogenetics induced axon regeneration [1].

The experimental workflow is shown in Fig. 1. There were 12 experimental conditions studied each with 3 biological replicates for a total of 36 samples used for analysis. The samples were split into three batches of 12plex TMT for quantitation. Protein from the optic nerves was extracted, digested, labeled with TMT (Thermo Scientific™) and pooled for mass spectrometry analysis. The experimental conditions and TMT labels are shown in Table 1.

Raw scans were acquired using a Q Exactive™ Orbitrap™ Mass Spectrometer (Thermo Scientific™) and analyzed using Proteome Discoverer™ 2.5 software. Protein data was normalized using densitometry and ImageJ. A two-way ANOVA followed by Tukey’s multiple comparisons test was performed using Graph Pad Prism version 8.0.0 for Windows (GraphPad Soft-
Fig. 1. Graphical abstract illustrating the experimental workflow from animal model preparation to software analysis and bioinformatics. Schematic created with BioRender.com.

Table 1
Table depicting the experimental conditions, grouping, and TMT labeling of the samples.

| TMT Tag | Biological Replicate 1 | Biological Replicate 2 | Biological Replicate 3 |
|---------|------------------------|------------------------|------------------------|
|         | Biological Replicate 1 | Biological Replicate 2 | Biological Replicate 3 |
|         | Sample Description     | Sample Description     | Sample Description     |
| 126     | WT 2WNC NS             | WT 2WNC NS             | WT 2WNC NS             |
| 127N    | WT 1WNC NS             | WT 1WNC NS             | WT 1WNC NS             |
| 127C    | WT 2WPC NS             | WT 2WPC NS             | WT 2WPC NS             |
| 128N    | WT 1WPC NS             | WT 1WPC NS             | WT 1WPC NS             |
| 128C    | Chr2 2WPC PS           | Chr2 2WPC PS           | Chr2 2WPC PS           |
| 129N    | Chr2 1WPC PS           | Chr2 1WPC PS           | Chr2 1WPC PS           |
| 129C    | Chr2 2WPC NS           | Chr2 2WPC NS           | Chr2 2WPC NS           |
| 130N    | Chr2 1WPC NS           | Chr2 1WPC NS           | Chr2 1WPC NS           |
| 130C    | Chr2 2WNC NS           | Chr2 2WNC NS           | Chr2 2WNC NS           |
| 131N    | Chr2 1WNC NS           | Chr2 1WNC NS           | Chr2 1WNC NS           |
| 131C    | Chr2 2WNC PS           | Chr2 2WNC PS           | Chr2 2WNC PS           |
| 132N    | Chr2 1WNC PS           | Chr2 1WNC PS           | Chr2 1WNC PS           |

Abbreviations: Chr2 = transgenic channelrhodopsin mice model. WT = wild-type control mice. 1WPC = 1 week post optic nerve crush. 2WPC = 2 weeks post optic nerve crush. 1WNC = 1 week no crush. 2WNC = 2 weeks no crush. PS = positive light stimulation. NS = no light stimulation.

The significant proteins found were used to perform a molecular function gene ontology (GO) with the PANTHER classification system (Fig. 2). The data was further analyzed to find the significant proteins in the channelrhodopsin post crush with stimulation (Chr2 PC PS) and no stimulation (Chr2 PC NS) conditions compared to the wild-type control (WT PC NS) (Table 2).
Table 2
Significant proteins identified in channelrhodopsin post crush no stimulation (Chr2 PC NS) and channelrhodopsin post crush positive stimulation (Chr2 PC PS) conditions, evaluating the effect of light stimulation on the crushed optic nerve.

| Accession Number / UniProt ID | Proteins | MW [kDa] | Ratios Chr2 PC NS | Ratios Chr2 PC PS | Protein Abundances Chr2 PC NS Sum | Protein Abundances Chr2 PC PS Sum | Protein Abundances WT PC NS Sum | Coverage [%] |
|-------------------------------|----------|----------|-------------------|------------------|-------------------------------|----------------------------------|----------------------------------|-------------|
| E0C230                        | Myosin light chain 1/3, skeletal muscle isoform (Fragment) | 13.5 | 1.2031 | 3.0501 | 167.7 | 425.8 | 139.6 | 9 |
| P68134                        | Actin, alpha skeletal muscle | 42 | 1.0698 | 2.9738 | 154.9 | 430.6 | 144.8 | 36 |
| Q546G1                        | Uncharacterised protein | 18.9 | 1.0227 | 2.4927 | 176 | 429 | 172.1 | 11 |
| Q90186                        | Uncharacterised protein | 32.8 | 1.1828 | 2.0661 | 161.1 | 281.4 | 136.2 | 21 |
| P32848                        | Parvalbumin alpha | 11.9 | 1.2482 | 1.9418 | 176 | 273.8 | 141 | 18 |
| Q5NC14                        | Fibrillar collagen NCI domain-containing protein (Fragment) | 28.8 | 1.1024 | 1.8199 | 204.5 | 337.6 | 185.5 | 4 |
| Q921G7                        | Electron transfer flavoprotein-ubiquinone oxidoreductase, mitochondrial | 68 | 1.1698 | 1.6342 | 213.6 | 298.4 | 182.6 | 5 |
| Q3U02J1                       | Uncharacterised protein | 21.3 | 0.9151 | 1.5722 | 176.7 | 303.6 | 193.1 | 14 |
| A0A046XK8                      | Nucleoside-diphosphate kinase (Fragment) | 10.6 | 1.4562 | 1.5317 | 204.6 | 215.2 | 140.5 | 24 |
| P13783                        | Cytochrome c oxidase subunit 4 isomeric 1, mitochondrial | 19.5 | 1.0835 | 1.5270 | 194.7 | 274.4 | 179.7 | 12 |
| D4N6R6                        | Beta-globin | 15.8 | 1.2337 | 1.4590 | 191.1 | 226 | 154.9 | 22 |
| A0A0484J05                    | Lamin-B2 | 69 | 1.0272 | 1.4540 | 219 | 310 | 213.2 | 6 |
| Q546G4                        | Serum albumin | 68.6 | 1.3703 | 1.4464 | 243.1 | 256.6 | 177.4 | 25 |
| Q6B822                        | Histone H4 (Fragment) | 4.9 | 0.9695 | 1.4358 | 178.4 | 265.6 | 183.7 | 42 |
| Q9SFH5G1                      | Nestin | 207 | 1.1029 | 1.4377 | 224 | 292 | 203.1 | 1 |
| Q36UX9                        | Rab GDP dissociation inhibitor | 50.5 | 1.2149 | 1.2442 | 199 | 203.8 | 163.8 | 9 |
| P63087                        | Serine/Threonine protein phosphatase PPI-gamma catalytic subunit | 37 | 1.4286 | 1.0609 | 244 | 181.2 | 170.8 | 3 |
| P07759                        | Serine protease inhibitor A3K | 46.9 | 1.3480 | 1.0000 | 213.8 | 158.6 | 158.6 | 9 |
| Q37U2E2                       | Fibrillar collagen NCI domain-containing protein (Fragment) | 116.8 | 1.4551 | 0.8718 | 212.3 | 127.2 | 145.9 | 1 |
| Q6A074                        | MKIAA4004 protein (Fragment) | 107.7 | 0.6940 | 0.7990 | 161.9 | 186.4 | 233.3 | 1 |
| A0A34Q41U0                     | Calmodulin-1 | 12.9 | 0.7506 | 0.7860 | 162.7 | 170.4 | 216.8 | 42 |
| Q92339                        | Phosphoelastin | 10.3 | 0.9215 | 0.7454 | 179.5 | 145.2 | 194.8 | 13 |
| Q80YU9                        | Retinoblastoma binding protein 9 | 20.9 | 0.7212 | 0.6412 | 175.9 | 156.4 | 243.9 | 4 |
| P14069                        | Protein S100-A6 | 10 | 0.7839 | 0.5395 | 216.5 | 149 | 276.2 | 17 |

Ratios were calculated by combining the grouped protein abundances from 1- and 2-weeks post crush for both the light stimulated and non-stimulated groups, respectively, and then dividing by the control group WT PC NS (created by combining 1-week and 2-week WT PC NS conditions). Proteins are sorted from highest abundance ratio to lowest abundance ratio in the Chr2 PC PS condition. Cells highlighted in green indicate an upregulation of protein, while cells highlighted in blue indicate a downregulation of protein in that same condition. Cells in yellow show a ratio of 1, indicating no upregulation or downregulation. The accession number listed is related to the UniProt ID and can be used to access individual protein information at [https://www.uniprot.org](https://www.uniprot.org).
Fig. 2. PANTHER Gene Ontology (GO) pie chart depicting the molecular functions of the significant proteins across all experimental conditions.

2. Experimental Design, Materials and Methods

2.1. Optic Nerve Crush and Light Stimulation

All animal procedures were approved and performed in accordance with the ARVO statement for the use of animals in ophthalmic and vision research and with the Animal Care and Use Committee at the University of Miami. Thy1-Chr2-eYFP and C57BL/6J mice were purchased from Jackson Laboratory (Stock Nos. 007615 and 000664). The transgenic Thy1-Chr2-eYFP mice express channelrhodopsin (Chr2), a light-gated, cation-selective ion channel isolated from the green algae Chlamydomonas reinhardtii. Chr2 is fused together with an enhanced yellow fluorescent protein (eYFP) that is controlled by the Thy1 promoter.

Thy1-Chr2-eYFP and C57BL/6J mice received optic nerve crush at 3-months of age. All animals were anesthetized by intraperitoneal (IP) injection of a ketamine/xylazine dilution. One drop of proparacaine was given to each eye to serve as a topical anesthetic and block local reflex. The left optic nerve was exposed through a small window made between the surrounding muscles. The optic nerve was crushed under visualization approximately 0.5–1 mm behind the globe with Dumont #5 forceps (Fine Science Tools, Foster City, CA, USA) for four seconds. Care was taken to prevent damage to the ophthalmic artery and minimize bleeding. Ophthalmic ointment containing neomycin (Akorn, Somerset, New Jersey, NJ, USA) was applied post-procedure to minimize risk of infection.

Mice were randomly assigned to either the optogenetic stimulation group or no stimulation group. The mice in the optogenetic stimulation group were subjected to 470 nm wavelength blue light stimulation at 1 Hz frequency. Blue light was delivered by a special mouse housing cage affixed with 20 blue light LEDs, each with an output of 10 mW (LED supply, 296 Beanville Road, Randolph, VT 05060). The frequency was controlled by a programmable digital cycle timer (Uctronics, Nanjing, China). The mice in the no light stimulation group were kept in a normal 12 h light/dark cycle. The mice in the experimental and control groups were sacrificed one or two weeks after crush according to the experimental design.
2.2. Protein Extraction, Digestion and Labeling

Optic nerve samples were carefully minced in a protein extraction buffer composed of 10 mM TEAB pH 8.5, 50 mM NaCl and 0.1% SDS. The samples were vortexed and centrifuged. Supernatants were then placed in a fresh tube. The procedure was repeated three times for optimal protein extraction. Dot blot densitometry and Imagej software were used to estimate and normalize protein amounts across all samples. Protein was denatured, reduced, and alkylated by 2% SDS, 110 mM TCEP and 84 mM iodoacetamide respectively. Samples were in-solution trypsin digested overnight with 10 μL of a 0.1 μg/μL concentration of trypsin and then labeled with TMT label reagents.

There were 12 experimental conditions used, each with three biological replicates. Each biological replicate was uniquely labeled using 12 tags from a modified 16plex labeling kit (Thermo Scientific) for a total of three batches. Each batch was combined into a newly labeled Eppendorf tube for mass spectrometry analysis. Samples were cleaned with PierceTM C18 Tips (Thermo ScientificTM) and speed vacuumed prior to LC-MS/MS analysis.

2.3. Untargeted Liquid Chromatography and Mass Spectrometry

Dried samples were reconstituted in 50 μL of 2% acetonitrile in water with 0.1% formic acid and then sonicated in an ultrasonic water bath for 15 min. Samples were then transferred to their respective autosampler vial. Proteins were separated using a Thermo ScientificTM Easy-nLC 1000 system and 1 μL of sample was injected onto an Easy-Spray HPLC column (Thermo ScientificTM ES900). The flow rate was 300 nL/min. Mobile phase A consisted of water with 0.1% formic acid (v/v) and mobile phase B was acetonitrile with 0.1% formic acid (v/v). The column temperature was 55 °C.

Samples were ionized and detected on an EASY-SprayTM source coupled to a Q ExactiveTM mass spectrometer (Thermo ScientificTM). Spray voltage was set to 1.9 kV and capillary temperature to 300 °C. S-Lens RF Level was set to 60.0. For full scan, mass range was 375–1400 m/z, resolution was 70,000, and microscans was 1. The AGC target was 3e6 and maximum inject time was 50 ms. For dd-MS2, mass range was 200–2000 m/z, resolution was 35,000, AGC target was 1e5, and maximum injection time was 100 ms. The instrument was set to Top 10, isolation window 1.2 m/z and the NCE to 32. The intensity threshold was 2.04e4 and dynamic exclusion was 30.0 s.

2.4. Data Processing

Proteins were identified from their Thermo.RAW files using Proteome Discoverer™ 2.5 software. The Mus musculus (mouse) proteome was downloaded from UniProt and used as the target database. Max missed cleavage sites was set to 2 and minimum peptide length to 6. Precursor Mass Tolerance was set to 10 ppm and Fragment Mass Tolerance to 0.02 Da. Post-translational modifications included oxidation, acetylation, carbamidomethylation, and TMTpro (304.207 Da). Normalization was set to total peptide amount and confidence to high. A 12plex TMT label reagent kit (modified from a 16plex Thermo Scientific kit) was set for quantitation. Proteome Discoverer initially identified 745 proteins, 378 with quantitative data. The mean of the three biological replicates (grouped abundances) and their coefficient of variations were uploaded into Graph Pad Prism 8.0.0. A two-way ANOVA was performed using Tukey’s multiple comparison test. The results were filtered to identify 24 significant proteins within the Chr2 PC PS and Chr2 PC NS conditions compared to control (WT PC NS).
**Ethics Statements**

All experiments were performed in compliance with the U.S. National Institute of Health Guide for the Care and Use of Laboratory Animals and the University of Miami IACUC approved protocol 20-098. This study utilized mouse models encompassing both genders. The sex of these mouse models is not known to influence or have an association with optic nerve regeneration.

**Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

**Data Availability**

Labeled quantitative proteomics dataset of optogenetics induced axon regeneration (Accession no. PXD32788) (Original data) (Proteomics Identifications Database).

**CRediT Author Statement**

Faith Christine Harvey: Investigation, Data curation, Formal analysis, Writing – review & editing; Ximena Mendoza: Writing – original draft; Yuan Liu: Investigation, Writing – review & editing, Resources; Richard K. Lee: Conceptualization, Methodology, Supervision, Writing – review & editing, Resources; Sanjoy K. Bhattacharya: Conceptualization, Methodology, Supervision, Writing – review & editing, Resources.

**Acknowledgments**

This work was partially supported by the U.S. Department of Defense Grant (DOD), W81XWH1910845, NIH Grant U01EY027257, NIH Core Grant P30EY014801, and an unrestricted grant from The Research to Prevent Blindness to the Bascom Palmer Eye Institute. RKL and part of this research is supported by the Walter G Ross Foundation. The mass spectrometry data has been deposited to the Proteome Xchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE partner repository with the dataset identifier PXD032788.

**Reference**

[1] J. Arcuri, Y. Liu, R.K. Lee, S.K. Bhattacharya, Lipid profile dataset of optogenetics induced optic nerve regeneration, Data Brief 31 (2020) 106001, doi:10.1016/j.dib.2020.106001.