Jun 06, 2025 Version 1

Liquid Chromatography-Mass Spectrometry (LC-MS) with striatal synaptosomes V.1

DOI
https://dx.doi.org/10.17504/protocols.io.eq2lyqk6mvx9/v1

Chuyu Chen[1], Loukia Parisiadou[1]

[1]Northwestern University, Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815

Chuyu Chen
Northwestern University, Aligning Science Across Parkinson's...

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.eq2lyqk6mvx9/v1

Protocol Citation: Chuyu Chen, Loukia Parisiadou 2025. Liquid Chromatography-Mass Spectrometry (LC-MS) with striatal synaptosomes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lyqk6mvx9/v1

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: June 06, 2025

Last Modified: June 06, 2025

Protocol Integer ID: 219671

Keywords: ASAPCRN, LC-MS, synaptosome, studies on striatal synaptosome, striatal synaptosome, pathogenic hyperactive lrrk2g2019s mutation exhibit deficit, exhibit deficits in dopamine release, pathogenic hyperactive lrrk2g2019, dopamine release, dopamine, mass spectrometry, synaptosome, quantitative phosphoproteomic liquid chromatography, striatum, underlying molecular mechanism, mice

Funders Acknowledgements:
Aligning Science Across Parkinson's [ASAP-020600] through the Michael J. Fox Foundation for Parkinson's Research (MJFF)
Grant ID: ASAP-020600

Abstract

We and others previously reported that knock-in mice expressing the pathogenic hyperactive Lrrk2^{G2019S} mutation exhibit deficits in dopamine release within the striatum. To investigate the underlying molecular mechanisms, we conducted quantitative phosphoproteomic Liquid Chromatography-Mass Spectrometry (LC-MS) studies on striatal synaptosomes from Lrrk2^{G2019S} mice.

Safety warnings

These protocols need prior approval by the users' Institutional Animal Care and Use Committee (IACUC) or equivalent ethics committee

LC-MS sample preparation

1 Mouse striata were dissected three hours after administering a dose of MLi-2 (10 mg/kg, 3hours) or control vehicle via oral gavage. The tissues were flash frozen and stored at −80°C until use

2 three mouse striata were pooled and rapidly homogenized in four volumes of ice-cold Buffer A (0.32 M sucrose, 5 mM HEPES, pH 7.4, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$) supplemented with a Halt protease and phosphatase inhibitor cocktail (Thermo) using a Teflon homogenizer with 12 strokes.

3 The homogenized brain extract was then centrifuged at 1400 g for 10 minutes. The supernatant (S1) was saved, and the pellet (P1) was homogenized in Buffer A with a Teflon homogenizer (five strokes).

4 After centrifugation at 700 g for 10 minutes, the supernatant (S1′) was pooled with S1.

5 The pooled S1 and P1were centrifuged at 13,800 g for 10 minutes, resulting in a crude synaptosomal pellet (P2) and its corresponding supernatant (S2).

6 The P2 pellet was resuspended in Buffer B (0.32 M sucrose, 6 mM Tris, pH 8.0), also supplemented with the protease and phosphatase inhibitor cocktail, using a Teflon homogenizer (five strokes).

7 P2 was then carefully loaded onto a discontinuous sucrose gradient (0.8 M/1 M/1.2 M sucrose solution in 6 mM Tris, pH 8.0) with a Pasteur pipette, followed by centrifugation in a swinging bucket rotor at 82,500 g for 2 hours.

8 The synaptic plasma membrane fraction (SPM) located at the interphase between the 1 M and 1.2 M sucrose fractions was collected using a syringe and transferred to clean ultracentrifuge tubes.

9 Samples were then centrifuged in a swinging bucket rotor at 200,000 g for 30 minutes. The supernatant was removed and discarded, while the SPM pellet was flash frozen and stored at −80°C.

LC-MS -The SPM pellet samples (processed by Tymora Analytical Operations in West Lafayette, Indianapolis)

10 For the lysis process, 200 µL of phase-transfer surfactant lysis buffer (PTS), which contains 12 mM sodium deoxycholate, 12 mM sodium lauroyl sarcosinate, 10 mM TCEP, and 40 mM CAA, was added to each tissue sample. A phosphatase inhibitor cocktail 3 (Millipore-Sigma) was also included.

11 The samples were incubated for 10 minutes at 95°C, pulse-sonicated several times, and then incubated for an additional 5 minutes at 95°C.

12 The lysed samples were centrifuged at 16,000 × g for 10 minutes to remove debris, and the supernatant was collected.

13 The samples were diluted fivefold with 50 mM triethylammonium bicarbonate, and a BCA assay was performed to determine protein concentration, normalizing all samples by protein amount.

14 The samples were then normalized to 300 ug protein in each and digested with 6 mg Lys-C (Wako) for 3 h at 37°C.

15 6 ug trypsin was added for overnight digestion at 37°C.

16 The supernatants were collected and acidified with trifluoroacetic acid (TFA) to a final concentration of 1% TFA. Ethyl acetate solution was added at 1:1 ratio to the samples. The mixture was vortexed for 2 min and then centrifuged at 16,000 × g for 2 min to obtain aqueous and organic phases.

17 The organic phase (top layer) was removed, and the aqueous phase was collected, dried completely in a vacuum centrifuge, and desalted using Top-Tip C18 tips (Glygen) according to manufacturer's instructions.

18 The samples were dried completely in a vacuum centrifuge and subjected to phosphopeptide enrichment using PolyMAC Phosphopeptide Enrichment kit (Tymora Analytical) according to manufacturer's instructions, and the eluted phosphopeptides dried completely in a vacuum centrifuge.

19 The full phosphopeptide sample was dissolved in 10.5 µl of 0.05% trifluoroacetic acid with 3% (vol/vol) acetonitrile and 10 µl of each sample was injected into an Ultimate 3000 nano UHPLC system (Thermo
Fisher Scientific).

20 Peptides were captured on a 2-cm Acclaim PepMap trap column and separated on a 50-cm column packed with ReproSil Saphir 1.8 µm C18 beads (Dr. Maisch GmbH). The mobile phase buffer consisted of 0.1% formic acid in ultrapure water (buffer A) with an eluting buffer of 0.1% formic acid in 80% (vol/vol) acetonitrile (buffer B) run with a linear 90-min gradient of 6–30% buffer B at flow rate of 300 nL/min. The UHPLC was coupled online with a Q-Exactive HF-X mass spectrometer (Thermo Fisher Scientific).

21 The mass spectrometer was operated in the
data-dependent mode, in which a full-scan MS (from m/z 375 to 1,500 with the resolution of 60,000) was followed by MS/MS of the 15 most intense ions (30,000

resolution; normalized collision energy - 28%; automatic gain control target (AGC) - 2E4, maximum injection time - 200 ms; 60sec exclusion].

MS Data analysis

22 The raw files were searched directly against the mouse database with no redundant entries, using Byonic(Protein Metrics) and Sequest search engines loaded into Proteome Discoverer 2.3 software (Thermo Fisher Scientific).

23 The data from the two search engines was combined. In most cases, the same peptides were identified by both. The final data reported is the combination of both search engines, including the identifications reported by only one search engine.

24 MS1 precursor mass tolerance was set at 10 ppm, and MS2 tolerance was set at 20 ppm. Search
criteria included a static carbamidomethylation of cysteines (+57.0214 Da), and variable modifications of phosphorylation of S, T and Y residues (+79.996 Da), oxidation (+15.9949 Da) on methionine residues and acetylation (+42.011 Da) at N terminus of proteins.

25 Search was performed with full trypsin/P digestion and allowed a maximum of two missed cleavages on the peptides analyzed from the sequence database. The false-discovery rates of proteins and peptides were set at 0.01.

26 All protein and peptide identifications were grouped and any redundant entries were removed. Only unique peptides and unique master proteins were reported.

27 All data were quantified using the label-free quantitation node of Precursor Ions Quantifier through the Proteome Discoverer v2.3 (Thermo Fisher Scientific).

28 For the quantification of phosphoproteomic data, the intensities of phosphopeptides were extracted with initial precursor mass tolerance set at 10 ppm, minimum number of isotope peaks as 2, maximum ΔRT of
isotope pattern multiplets – 0.2 min, PSM confidence FDR of 0.01, with hypothesis test of ANOVA, maximum RT shift of 5 min, pairwise ratio-based ratio calculation, and 100 as the maximum allowed fold change.

29 For calculations of fold-change between the groups of proteins, total phosphoprotein abundance values were added together and the ratios of these sums were used to compare proteins within different samples.

30 Threshold forsignificance was set to $p<0.05$ (unadjusted p-value) & FC (difference on the log2 intensity values) ≥ 0.58.