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LC-MS of Native Nanodiscs V.1

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Caroline Brown^{1,2,3}, Snehasish Ghosh^{1,2,3}, Rachel McAllister^{1,2,4}, Mukesh Kumar^{5,6}, Gerard Walker^{1,2,4}, Eric Sun¹, Talat Aman¹, Aniruddha Panda^{1,2}, Shailesh Kumar⁴, Wenxue Li^{4,7}, Jeff Coleman^{1,2}, Yansheng Liu^{4,7}, James E Rothman^{1,2}, Moitrayee Bhattacharyya⁴, Kallol Gupta^{1,2,3}

¹Nanobiology Institute, Yale University, West Haven, CT, USA;

²Department of Cell Biology, Yale University School of Medicine, New Haven, CT, USA;

³Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815;

⁴Department of Pharmacology, Yale University, New Haven, CT, USA;

⁵F.M. Kirby Neurobiology Center, Department of Neurobiology, Boston Children's Hospital, Harvard Medical School, Boston, MA 02115, USA;

⁶Cell Signaling Technology, Inc., Danvers, MA 01923, USA;

⁷Yale Cancer Biology Institute, Yale University, West Haven, CT, USA



Caroline Brown

Yale University

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol provides a step-by-step guide for conducting LC-MS analysis of native nanodiscs extracted from biological membranes.

Liquid Chromatography

- 1 Resuspend peptides in water + 0.1 formic acid varying amount of water so that 500ng of peptide can be loaded in a reasonable volume.
- 2 Chromatography is subsequently conducted using home-packed C18 columns (15cm x 75uM ID) for separation of peptides by hydrophobicity.
- 3 Use a 75 minute separation gradient with a buffer system of water/acetonitrile with 0.1% formic acid beginning at 6% acetonitrile and ending at 100% acetonitrile.

Mass Spectrometry

- 4 Data Dependent Acquisition (DDA) mass spectrometry is conducted using an Orbitrap Eclipse instrument.
- 5 MS1 scans are acquired using the Orbitrap detector at a resolution of 120000 and stored in profile mode using a mass range of 350-1400 m/z and an intensity threshold of 1.0e3.
- 6 Use a cycle time of 1 second for ion isolation and MS2 fragmentation isolating ions with a 1.4 m/z window and fragmented with CID energy at 30% activation.
- 7 Product ions are detected at the ion trap set to rapid detection mode with precursor ions being dynamically excluded for 20 seconds after one instance of detection.