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LC-MS of Native Nanodiscs V.2

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Caroline Brown[1,2,3], Snehasish Ghosh[1,2,3], Kallol Gupta[1,2,3]

[1]Nanobiology Institute, Yale University, West Haven, CT, USA;
[2]Department of Cell Biology, Yale University School of Medicine, New Haven, CT, USA;
[3]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815

C Caroline Brown
Yale University

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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 a 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 and 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 precursors ions being dynamically excluded for 20 seconds after one instance of detection.