Network Biology Collaborative Centre

Sinai Health | Lunenfeld-Tanenbaum Research Institute

NBCC Proteomics Guide

The Network Biology Collaborative Centre (nbcc.lunenfeld.ca) at the LTRI provides services for the identification and quantificaton of proteins in a state-of-the-art facility. This guide provides information on the proteomics applications we offer and the instrumentation that is available for analysis.

I. Who we are

The NBCC Proteomics node is overseen by Sinai Health's Vice-President of Research and NBCC co-Director Dr. Anne-Claude Gingras who is an expert in cellular signaling and protein-protein interaction proteomics. The Scientific Manager Dr. Brendon Seale, with over 10 years' experience in mass spectrometry focused on micro-sized sample processing and ion mobility, leads analytical assay development. Cassandra Wong, with 5-plus years of expertise in protein interaction mapping, manages the day-to-day operations of the facility handling a myriad of projects from inception to completion. They are assisted by technicians Laura McGary and Zhen Lin. Laura's field of expertise lies in chemical proteomics, while Zhen has over 15 years' experience in protein-protein interaction proteomics. The NBCC Director of Operations Dr. Karen Colwill provides administrative and logistical support.

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Research Resource Identifier (RRID): SCR_025375

II. How we help

The NBCC provides full support for your proteomics needs from initial study concept to manuscript preparation and data submission to public repositories. Our service starts with an initial consultation to identify the best and most cost-effective approach to your project. Our services include sample preparation, quality control, mass spectrometry analysis, data analysis support, upload of data into public repositories, and assistance with grant and publication writing related to the services we offer.

III. Applications

We support and offer proteomics applications that range from measuring purified proteins to quantifying the proteome of cells and tissues.

A. Protein-protein interaction or proximal protein mapping

We are specialists in protein-protein interaction mapping. We offer reagents, expertise, and a data analysis pipeline to identify interacting proteins (immunoaffinity purification (e.g., FLAG tag)) and proximal proteins (proximity-dependent biotinylation (PDB) methods (often referred to as BioID)). These assays can be performed re-iteratively for proteins in the same pathway or protein family to create maps of signaling networks.

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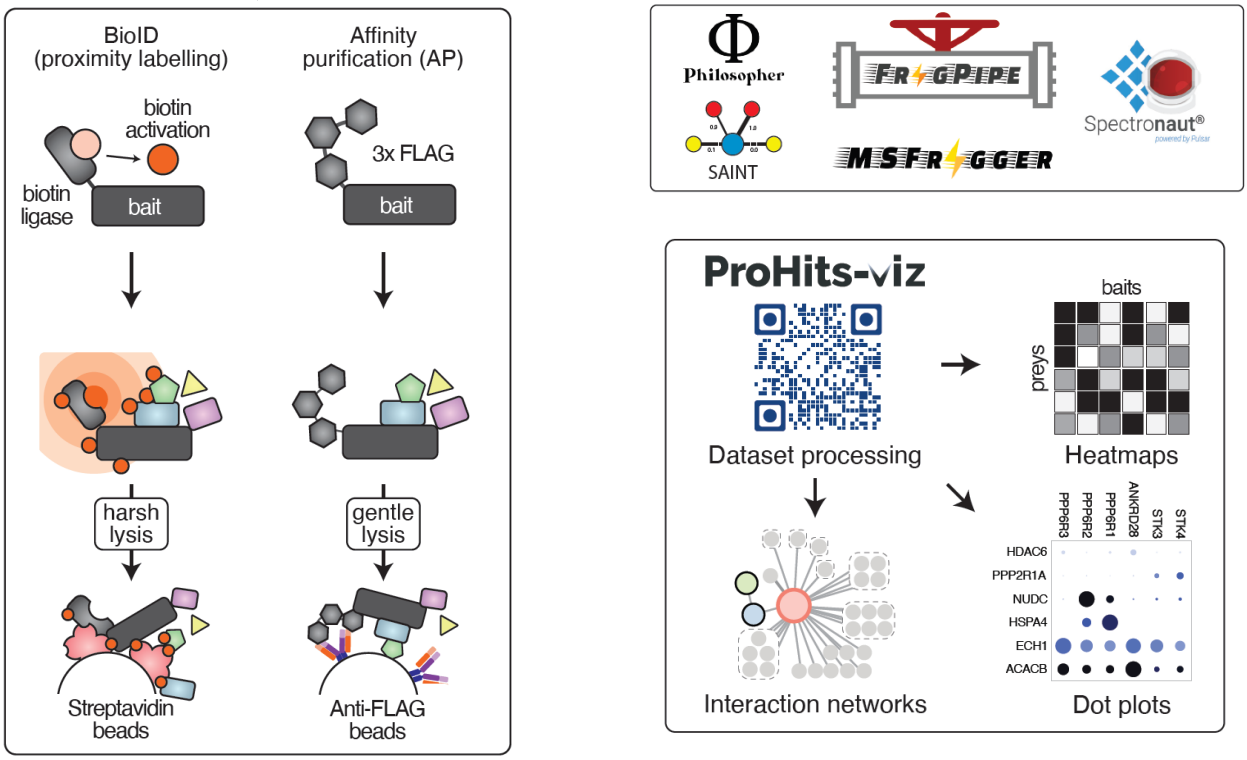


Figure 1: Protein-protein interaction workflow. Proteins of interest (bait) are fused to an abortive biotin ligase (for PDB/BioID) or 3xFLAG (for immunoaffinity purification). For PDB, nearby proteins are biotinylated, and cells undergo harsh lysis. Biotinylated proteins are enriched with streptavidin beads. For FLAG-AP, protein-protein interactions are maintained with a gentle lysis and enriched with anti-FLAG beads. Tools such as Fragpipe, MSFragger, Spectronaut, Philosopher, and SAINT are used to search and analyze the data generated by the mass spectrometer. ProHits-viz is used to visualize the results through heatmaps and dot plots or prepares data for export to Cytoscape for interaction network mapping.

B. Differential proteome abundance

We routinely perform global proteome analysis to determine differences between cell states from cell lines, complex tissue, or biofluids. We comprehensively track changes between different populations (e.g., normal vs disease) or treatments. To achieve high protein coverage, we identify and quantify proteins from these complex mixtures in an unbiased manner on our state-of-the-art instruments (e.g., timsTOF or Orbitrap Astral).

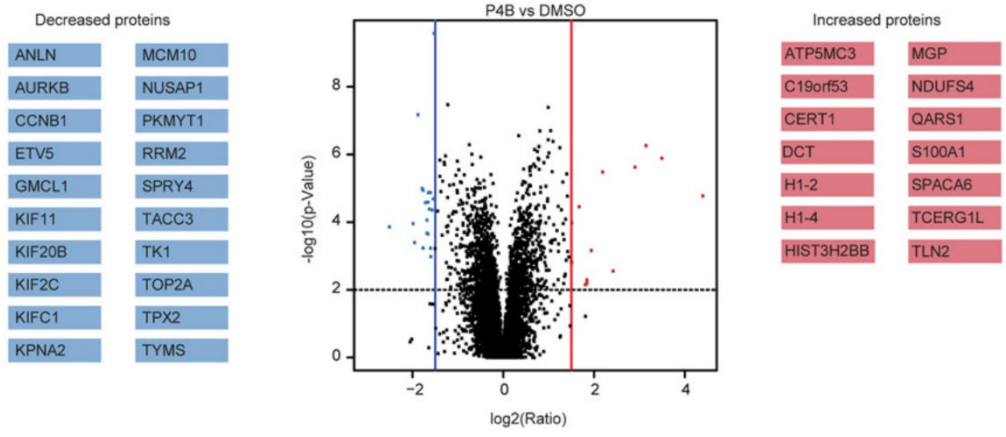


Figure 2: Differential proteome abundance upon treatment with a PROTAC. A375 cells were treated for 24hr with 200 µM of P4B (PROTAC) or DMSO and proteins were measured with an Orbitrap Fusion™ Lumos™ Tribrid™ (Posternak et al., Nat Chem Biol (2020)).

Blue and red vertical lines are log2 fold change cutoffs of +/- 1.5. The dotted horizontal line represents a p-value of 0.01. Significantly changing proteins are listed and coloured in the figure.

C. Phosphoproteome

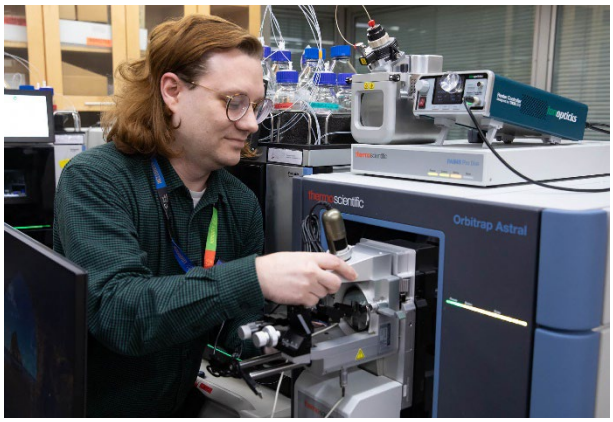
Protein phosphorylation is a primary mechanism through which signaling cues are transmitted within a cell. We enrich phosphopeptides from whole lysate to map differences between conditions using differential abundance analysis. Although phosphorylation analysis is our most popular service, mass spectrometry can be applied to map other post-translational modifications. Please inquire about your PTM of interest.

D. Chemical proteomics

Assessing target engagement is a key step in the drug development process. We offer pull-downs and competition assays to identify which proteins bind small molecules or biologics. These tests enable the rapid assessment of on- and off-target binding during early-stage drug validation.

IV. Core instrumentation

The NBCC provides proteomics services using 5 mass spectrometers from 3 different vendors: Thermo, Bruker, and Sciex.



All Thermo instruments are compatible with quantitative approaches such as data-independent acquisition (DIA, global analysis), parallel-reaction monitoring (PRM, targeted analysis), and multiplexing using isobaric tandem mass tags (TMT).

Most notably, in 2023, we acquired a Thermo's Orbitrap Astral. The Orbitrap Astral excels when the highest degree of proteome coverage and sensitivity is required. It enables the identification of 8000 proteins from a single sample with a throughput of 80 samples per day.

Thermo's Fusion Tribrid Lumos offers a variety of workflows, including confident identification of post-translational modifications and the ability to tease apart complex cross-linked mixtures. The Lumos' capabilities are supported by electron transfer dissociation (ETD) and MSn fragmentation.

Bruker mass spectrometers coupled to Evosep Ones (HPLCs) enable robust and fast acquisition of samples by utilizing trapped ion mobility technology coupled to parallel accumulation serial fragmentation (PASEF).

Bruker's timsTOF Pro 2 is our workhorse for standard workflows such as protein-protein interaction and global proteome studies, enabling the identification of 6000 proteins per sample at a rate of 30 samples per day.

Bruker's timsTOF SCP is a single cell proteomics solution, enabling detection of 4000 proteins from a single-cell level sample (250 pg) and can process 40 samples per day.

We have one Sciex TripleTOF 6600 which is geared towards the identification of protein complexes and protein-protein interactions.

In addition to mass spectrometers, we have an Opentrons Flex liquid handler to empower and facilitate high-throughput proteomics studies.