

Introduction

The Proteomics Resource Center (PRC) at The Rockefeller University offers mass spectrometrybased analysis of a vast variety of molecules for The Rockefeller University community on a subsidized, cost-recovery basis. Based on capacity, the Center also work with other academic and non-profit institutions. The Center is currently not open to forprofit organizations. The PRC is located on The Rockefeller University campus in the Rockefeller Research Building, RRB157.



The Proteomics Resource Center (PRC) is located on the first floor of the Rockefeller Research Bldg. (RRB) - marked by the red dot.

Our Mission

The primary goal of the Center is to help research groups of The Rockefeller University applying analytical mass spectrometry tools towards answering biological questions. The goal of the Center is also to extend the limits of sensitivity and broaden the range of available analytical technologies.

Our Expertise

The Center have expertise in identification, characterization and quantitation of proteins, peptides, metabolites, lipids and small molecules (e.g drugs) by liquid chromatography and mass spectrometry. The Center is equipped with nanoflow and higher flow liquid chromatography systems and mass spectrometers capable of measuring molecules with high mass accuracy and high resolution. The Center is also equipped with, and has expertise in using a wide array of software for data analysis. Though the Center is named the

'Proteomics Resource Center' many samples analyzed by the Center are not directly related to proteins and peptides. For example, the past years the Center has dedicated resources to establish a Metabolomics Platform that can measure more than 200 polar metabolites using high mass accuracy/high resolution targeted LC-MS. The analysis of lipids by LC-MS/MS, often in discovery mode, has also been implemented and is a tool offered to our users.

Boutique Analysis

Yearly, the Center receives 500+ requests from more than 80 laboratories. The questions that our collaborators/users asks are nearly always about identification and quantitation. Though the questions are the same, expectations to and the premises for the experiments are often very different. To understand which of the many tools and techniques we have available that will be most appropriate for a particular question it is important that we understand details related to a project. This makes it important to discuss a project with the project owner so that we together can devise a strategy for a successful In addition to getting a clear idea of analysis. expectations such meetings are also key to devising a workflow that can bring the molecules to be measured from the biological matrix to a buffer that is compatible with liquid chromatography mass spectrometry. With the latter in mind we has composed a series of guidelines of how to prepare the samples most commonly encountered by our Center for an LC-MS/MS analysis.



The scientists of the Proteomics Resource Centerat The Rockefeller University.



...continued

But the contact between the PRC and the user does not necessarily stop here: after data are generated the PRC team is often working closely with the user giving input on how to read the data, help to best present the data, or help generating figures and tables for grants and publications.

All of the above is possible because the PRC team is composed of scientists with backgrounds in analytical mass spectrometry and chemistry combined with the large exposer to many very different type of projects analyzed by the Center. A very large amount of knowledge is concentrated in the Center which makes it possible to tailor an analysis to a question and provide a boutique analysis.

While the PRC team is strong in analytical techniques we have less hands-on-experience related to how (biological) samples are generated. However, we do have knowledge of which labs and users that are using a specific sample generating techniques which makes it possible for the PRC to act as 'match-makers' between an experienced user and a user who wants to try a new tool - of course, only when possible, appropriate and with consent.

The PRC team

Typical Requests

- Exosome profiling [1]
- Co-immunoprecipitations [2]
- Targeted quantitation [3]
- SILAC based quantitation [4]
- Characterization of cyclic peptides [5]
- Nucleoside analysis [6]
- Analysis of HLA-I peptides [7]
- Post translational Modifications [8, 9]
- Protein complex characterization [10]
- Tandem Mass Tag based quantitation [11]
- Polar metabolite and lipid profiling [12]

References

- 1. Hoshino, A., et al., *Tumour exosome integrins determine* organotropic metastasis. Nature, 2015. **527**(7578): p. 329-35.
- 2. Liu, K., et al., *PI31 Is an Adaptor Protein for Proteasome Transport in Axons and Required for Synaptic Development.* Dev Cell, 2019.

- 3. Simon, D.J., et al., Axon Degeneration Gated by Retrograde Activation of Somatic Pro-apoptotic Signaling. cell, 2016.
- 4. Maze, I., et al., *Critical Role of Histone Turnover in Neuronal Transcription and Plasticity*. Neuron, 2015. **87**(1): p. 77-94.
- 5. Hover, B.M., et al., *Culture-independent discovery of the* malacidins as calcium-dependent antibiotics with activity against multidrug-resistant Gram-positive pathogens. Nat Microbiol, 2018.
- 6. Zhu, X., et al., *Role of Tet1/3 Genes and Chromatin Remodeling Genes in Cerebellar Circuit Formation*. Neuron, 2015.
- 7. Oh, C.Y., et al., *ALK and RET Inhibitors Promote HLA Class I Antigen Presentation and Unmask New Antigens within the Tumor Immunopeptidome*. Cancer Immunol Res, 2019. 7(12): p. 1984-1997.
- Govek, E.E., et al., Cdc42 Regulates Neuronal Polarity during Cerebellar Axon Formation and Glial-Guided Migration. iScience, 2018. 1: p. 35-48.
- 9. Garzia, A., et al., *The E3 ubiquitin ligase and RNA-binding protein ZNF598 orchestrates ribosome quality control of premature polyadenylated mRNAs.* Nat Commun, 2017. **8**: p. 16056.
- 10. Chaker-Margot, M., et al., *Stage-specific assembly events of the* 6-MDa small-subunit processome initiate eukaryotic ribosome biogenesis. Nat Struct Mol Biol, 2015.
- 11. Zhu, X.G., et al., *Functional Genomics In Vivo Reveal Metabolic Dependencies of Pancreatic Cancer Cells.* Cell Metab, 2020.
- 12. Soula, M., et al., *Metabolic determinants of cancer cell sensitivity* to canonical ferroptosis inducers. Nat Chem Biol, 2020.



Acknowledgments

The Proteomics Resource Center at The Rockefeller University acknowledges funding for mass spectrometer instrumentations from the **Sohn Conferences Foundation** and the **Leona M. and Harry B. Helmsley Charitable Trust**.