FROM THE DIRECTOR’S DESK

The use of high resolution and sensitive mass spectrometers, such as the Q-Exactive HF-X available at IMSU, allows going beyond protein identification into quantitation of proteome changes. There are two general methods use on quantitative proteomics, tandem mass tagging (TMT) and label-free quantitation (LFQ). Like any experimental approach, each has its advantages and limitations. TMT generates more precise quantitative data with less missing values, but this method is sensitive to the efficiency of the labeling step in sample preparation and it is more expensive. In contrast, LFQ does not require a labeling step, but the quantitative data is less precise due to more missing values. Nevertheless, Steven Gygi’s group recently compared both methods side by side, reaching the conclusion that both produce comparable accurate quantitative data [J. Proteome Res 2018, 17:1934-1942]. Regardless of the method used, accuracy and proteome depth depends on sample preparation efficiency and consistency. At IMSU, we would like to hear about your research goals and expectations to better assess how the available technology and developed analytical pipelines could contribute to reaching those goals. Please do not hesitate to reach out to schedule a meeting. Happy Holidays!!

TECHNICAL DISCUSSION – PEPTIDE LABELING

Label-free quantitation is the go-to choice for most proteomic experiments. LFQ algorithms vary between programs, but all function on the core principle that peptide chromatographic area is used as a representation of peptide quantity. This area is then compared with the peptide areas in sequential analysis, and the difference in area represents the change in peptide quantity. Protein quantities are then inferred through combination of all the peptide areas belonging to a single protein. In LFQ methods, the total protein content of each sample is carefully controlled in order to prevent artificial changes in peptide quantity. The peptide areas are normalized to the total protein content prior to quantitation.

Alternatively, peptide quantitation can be accomplished through labeling techniques. There are a large variety of labels available for purchase, and each uses a unique labeling chemistry. The simplest, and most commonly employed labeling method adds a fixed mass modification to specific amino acids on one sample, while a second, slightly heavier, fixed mass is added to a second sample. The difference is mass is accomplished by incorporating heavy carbon or deuterium isotopes into one of the labels. In this way, two different conditions can be mixed together and analyzed simultaneously while remaining distinguishable by the mass spectrometer. The difference in signal strength between two equivalent peptides is indicative of the change in protein quantity between conditions.
While isotopic labels work very well, they are not the correct choice for all experiments. Isotopic labels are normally restricted to two or three simultaneous conditions and have reduced performance in complicated samples with a wide dynamic range of proteins. More appropriate are isobaric labels, such as TMT tags, which are capable of simultaneously analyzing up to 11 different conditions. In isobaric labeling, each condition receives a modification of the same mass, but these modifications fragment differently in the MS2 spectrum. MS2 fragment intensities are then directly compared to determine relative protein quantitation.

ANNOUNCEMENTS

Congratulations to Scott Rothbart and Evan Cornett! Their work was highlighted in EurkAlert. https://www.eurekalert.org/pub_releases/2018-11/vari-hpe112718.php

We look forward to continuing this productive collaboration.

New email address and webpage. Now you can use the following email address, MSProt@msu.edu, and visit our website for more information at translationalscience.msu.edu/proteomics.

IMSU SUGGESTED ARTICLES

Here are some brand new publications demonstrating the abilities of labeled and label-free MS proteomics!

- "iTRAQ Based Quantitative Proteomics Approach Identifies Novel Diagnostic Biomarkers that were Essential for Glutamine Metabolism and Redox Homeostasis for Gastric Cancer" – PROTEOMICS - Clinical Applications, (2018)
- "Quantitative proteomic analysis of intracerebral hemorrhage in rats with a focus on brain energy metabolism" – Brain and Behavior, (2018)

Headway is being made on coupling laser capture microdissection to MS protein identification!


TRAINING

We offer training in mass spectrometry operations and sample preparation. See our website, and we can make arrangements to schedule a time that works for you.