BASIC OVERVIEW

The NMR-Based Metabolomics Core provides state-of-the-art technology in a centralized location accessible to research investigators and clinicians, serving CCHMC and UC COM communities as well as external academic and industry partners. The NBMC facilitates broad spectrum and targeted metabolomics analysis of polar components, as well as methods for targeted analysis of metabolites, with experience in the analysis of cells, organ tissue (e.g. liver, muscle, intestines, tongue, and tumor), biological fluids (e.g. urine, serum, plasma, amniotic fluid and saliva), and exhaled breath collected from human subjects or animal models.

The Metabolomics Facility is fully equipped to study multiple aspects of NMR, including ^{1}H, ^{13}C, and two-dimensional studies. We have performed expanded quantitative metabolic analyses on the following:

- Cells extracts/Cell secretions (media)
- Human and animal tissues and biopsy extracts
- Body fluids (blood, plasma, serum, urine, amniotic fluid, saliva, etc.)

The NBMC facility, located within the Division of Pathology at CCHMC, utilizes a Bruker IVDr 600MHz NMR system that is fully-automated, designed specifically for metabolomics based studies of biological fluids. The spectrometer utilized by the NBMC provides a high field multi-nuclear NMR capability to CCHMC, UCCOM and external academic and industry collaborators. The Bruker IVDr, a top-of-the-line spectrometer, is capable of running most contemporary homonuclear, heteronuclear one and two-dimensional pulse sequences, using pre-designed software, and is equipped with a 96-well SampleJet auto-sampler unit that renders the NMR system fully-automated. The Bruker spectrometer is equipped with a 5mm triple resonance conventional probe, which allows for routine acquisition of 1D and 2D ^{1}H-^{1}H correlations as well as 1D ^{13}C and 2D ^{1}H-^{13}C correlations used to confirm small metabolite identities. The usual array of peripheral analytical equipment is available including an electronic balance, pH meters, distillation equipment, bench-top centrifuges, rotary evaporators, a dual SpeedVac concentrator and lyophilizer system, and a cryogenic homogenizer.

GRANT BOILERPLATE

The NMR-Based Metabolomics Core (Director, Dr. Lindsey Romick-Rosendale) provides state-of-the-art technology in a centralized location accessible to research investigators and clinicians, serving CCHMC and UC COM communities. The NMR-based Metabolomics Core facilitates broad spectrum and targeted metabolomics analysis of polar components, as well as methods for targeted analysis of metabolites, with experience in the analysis of cells, organ tissue (e.g. liver, muscle, intestines, tongue, and tumor), biological fluids (e.g. urine, feces, serum, plasma, amniotic fluid and saliva), and exhaled breath collected from human subjects or animal models. The NBMC Facility measures concentrations of small molecules in biological samples. We use Bruker Biospin licensed TopSpin and Amix softwares in conjunction with Chenomx NMR Suite for analysis of unbiased metabolomics data. In short, proton (^{1}H) NMR spectra of all study groups are compared and analyzed using automated methodologies employing both univariate and multivariate statistical tools such as analysis of variance (ANOVA), significant difference spectra (SDS), principal component analysis (PCA) and partial least squares (PLS)(1).

Metabolite assignments will be made using one-dimensional ^{1}H NMR and two-dimensional ^{1}H-^{13}C and ^{1}H-^{1}H NMR experiments. Metabolites will be assigned by manual comparison with spectra of a reference library of molecules (Chenomx NMR Suite, http://www.chenomx.com/), as well as libraries available through the Human Metabolome Database (HMDB), the Biological Magnetic Resonance Data Bank (BMRB) and in-house standard samples. Pathway analysis and

time series analysis (MetaboAnalysts 3.0) can be utilized following identification of significant metabolites to determine the metabolic pathways altered. Quantification of metabolites will be performed by utilizing the previously noted software programs. As stated, the Chenomx NMR Suite software, as well as peak integration techniques, allows for absolute quantification of metabolites in biological specimens.

STABLE ISOTOPE-RESOLVED METABOLOMICS (SIRM)

Our newest capabilities established in collaboration with Dr. Andrew Lane at the University of Kentucky Resource Center for Stable Isotope-Resolved Metabolomics aims to pair global (untargeted) metabolic profiling with atom-resolved tracing of metabolites during metabolic transformations within cells, tissue or whole organisms. Investigators submit specific sample types with a metabolite that is enriched at all or any number of atoms with a stable isotope (such as ^{13}C or ^{15}N with natural abundances 1.1% and 0.37%, respectively), and the products are analyzed by NMR at various times following treatment. Both the isotopologue distributions and specific isotopomers in the different product metabolites are calculated, along with the total amounts of the metabolites. Taken together, this data provides detailed information about the relative importance of both parallel and interconnecting pathways.

NMR spectroscopy is sensitive, selective and can offer a vast amount of information about the degree to and which atoms are enriched from a precise precursor, even within a complex mixture. Standard one-dimensional NMR experiments routinely measure the total amount of a number of metabolites under steady state conditions, and cannot account for changes that the cell undergoes during the course of the experiment, such as increases in cell mass and subsequent elevations in the number of enzymes present in the cell. While these experiments are critical for future SIRM experiments, measuring input and output alone is not sufficient to explain specific intracellular network dynamics. Stable isotope-resolved metabolomics is critical for tracing pathway and fluxes in cells, animals and humans in their natural state. SIRM is complementary to other –omics technologies being utilized clinically, insight into metabolic dysfunction by SIRM can ultimately be integrated with genomic and proteomic information to attain systems biochemical insights in both model systems and specific human subjects. This knowledge is fundamental for transforming biomarker candidates into clinical practices.