

700 MHz NMR Spectroscopy Services on the North Carolina Research Campus Metabolomics, ¹³C-Tracing, Metabolite Identification and Quantitation



The Metabolomics and Exposome Laboratory (MEL) at the UNC Chapel Hill Nutrition Research Institute provides metabolomics, ¹³C-tracing, metabolite identification, and metabolite quantitation using a Bruker Avance III 700 MHz NMR spectrometer. We determine the presence and relative quantities of metabolites (e.g., host metabolites, host-gut microbiome co-metabolites) that are associated with disease, dysfunction, response to treatment (e.g., drugs, medications, chemicals), or diet related intake (e.g., supplements, nutrients, diets). NMR services are also available for tracking isotopically labeled compounds, elucidation of xenobiotic metabolism, compound identification, and quantitation.

Biospecimen Types. We have experience with a range of biospecimens (e.g., urine, plasma, serum, stool, tissues and cell extracts) in studies of osteoarthritis, cancer, obesity, liver and kidney disease, and addiction.

Metabolomics: Metabolomics investigations can be conducted using an approach where ¹H NMR spectra acquired for each study sample are binned, and statistical approaches are used to determine bins that associate with the study phenotype (e.g., case vs control, exposed vs unexposed, progressors versus non-progressors). Peaks within these bins are matched to a concentration pH sensitive library of over 330 standards (Chenomx NMR Suite 9 Professional). Specific sets of analytes (e.g., branched chain amino acids) can be selected for quantitative targeted analysis. NMR metabolomics can also be used to screen food, beverages, and supplements for purity.

Statistics, Multivariate Analysis, and Modelling. Descriptive statistics and hypothesis testing are conducted using R or SAS 9.4 (SAS Institute Inc., Cary, NC). Unsupervised (e.g., principal component analysis) and supervised (e.g., orthogonal partial least squares discriminate analysis, OPLS-DA) multivariate analysis of the normalized binned NMR data are conducted using SIMCA 17.0 (Sartorius Stedim Data Analytics, Umeå, Sweden). Bins with VIP ≥ 1 with low variance, or p-value and fold change of significance are considered important for differentiating study phenotypes. The Variable Importance to Projection (VIP) statistic from an OPLS-DA analysis is provided as the importance of the metabolites in differentiating the phenotypic groups. All models use a 7-fold cross-validation to assess the predictive variation of the model (Q²). Regression modelling can be performed using subject characteristic data, metabolite or bin data, and study outcome.

Pathway and Multi-omics analysis: MetaCore software (Clarivate Analytics, PA) or Metaboanalyst are used for pathway enrichment analysis, and pathways are ranked by p-value based on the hypergeometric test, which represents the enrichment of certain metabolites. Multi-omics data analysis is conducted by combining metabolomics with other omics (e.g., proteomics) using Metacore or Metaboanalyst.

Compound identification: NMR spectroscopy is a powerful technique for structure elucidation of both known or unknown molecules using 1D and 2D/multidimensional techniques such as COSY, HSQC, HMBC, TOCSY, and HSQC-TOCSY.

Tracing ¹³C-labeled compounds: Use of isotopically labeled compounds (e.g., ¹³C glucose, ¹³C glutamine) is a powerful method for tracing the fate of metabolites in pathways. Studies can be conducted using cells treated with ¹³C-labeled compounds, as well as for analysis of biological fluids or tissues obtained from humans or model systems administered the isotopic compounds.

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Citations

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