MICHIGAN STATE UNIVERSITY

Mass Spectrometry and Metabolomics Core Newsletter

Quarterly MSMC newsletter 2015 Issue 1

Dr. Tony Schilmiller welcomed as new Assistant Core Manager

Tony received his B.S. in biochemistry from Purdue University and performed undergraduate research on phenylpropanoid metabolism in Arabidopsis. He received his Ph.D. from Michigan State University in 2005 studying the biosynthesis of jasmonic acid and other oxylipins in tomato and Arabidopsis in the lab of Dr. Gregg Howe. In 2006, Tony joined the lab of Dr. Robert Last at MSU where his postdoctoral work focused on understanding specialized metabolism in tomato trichomes. During his postdoctoral research, Tony developed strong expertise in untargeted metabolite profiling (among many other things), and was a frequent user of the Mass Spectrometry and Metabolomics Core. He joined the Core as Assistant Manager in September 2015.

New e-mail contact

The Core has a new e-mail address to which all Core staff members have access. We encourage you to contact us at:

MassSpec.rtsf@cns.msu.edu

Instrument and software updates

Water Damage and Replacement of QTof LC/MS/MS

As many of you may know, one of the Core's high-resolution Q-Tof LC/MS/MS instruments (the G2-S QTof) was damaged beyond repair in July from water damage following a severe storm that sent water flooding into the Biochemistry basement. The good news is that this Waters G2-S Q-Tof has been replaced by a newer G2-XS Q-Tof model that offers improved sensitivity, mass resolution, and mass accuracy, and is nearly identical to the Waters G2-XS Q-Tof LC/MS/MS that was installed in March 2015. G2-XS #1 will continue to be used for metabolomics analyses, particularly those that employ ion-pairing reagents for LC/MS analyses of acidic water-soluble metabolites and oligonucleotides. The new G2-XS #2 is to be kept free of ion-pairing reagents and will be used for high-resolution MS for synthetic samples, analyses of intact proteins and peptides, hydrogen/deuterium exchange (HDX)

analyses of proteins, and metabolomics/lipidomic profiling where users want to avoid seeing ion-pairing reagent signals in their spectra. Installation of the new QTof was completed on October 28. Those wishing to be trained to operate the instrument should contact us at MassSpec.rtsf@cns.msu.edu.

New Triple-quadrupole LC/MS/MS

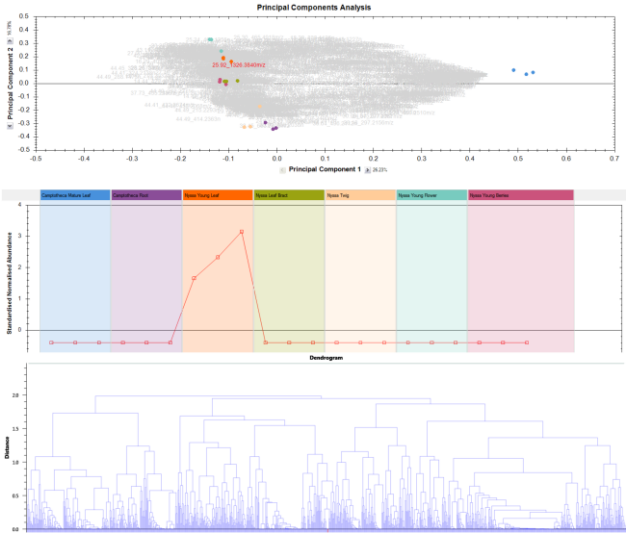
In addition to the Waters Quattro Micro API, Quattro Premier XE and Xevo TQ-S, and SCIEX QTRAP 3200, a fifth triple-quadrupole LC/MS/MS, the Waters TQ-D, will be commissioned in early November. This instrument will be equipped with a Waters Acquity UPLC pump and high-throughput PAL autosampler, and will allow for increased Core capacity for targeted analysis of metabolites using LC/MS/MS.

GC/MS Updates

Earlier this year, the Core replaced the aging Agilent 5973 GC/MS systems (Instruments A and Z) with a newer model 5975 single quadrupole instrument. Both of the Agilent GC/MS systems now have CTC-PAL autosamplers which offer improved performance and flexibility of operation. The Core now has four GC/MS instruments – three single-quadrupole (low resolution) instruments and a Waters GCT Premier time-of-flight high resolution instrument for accurate mass analyses. For more information on instruments, sample submission, and costs of running samples, see our website at: https://rtsf.natsci.msu.edu/mass-spectrometry/.

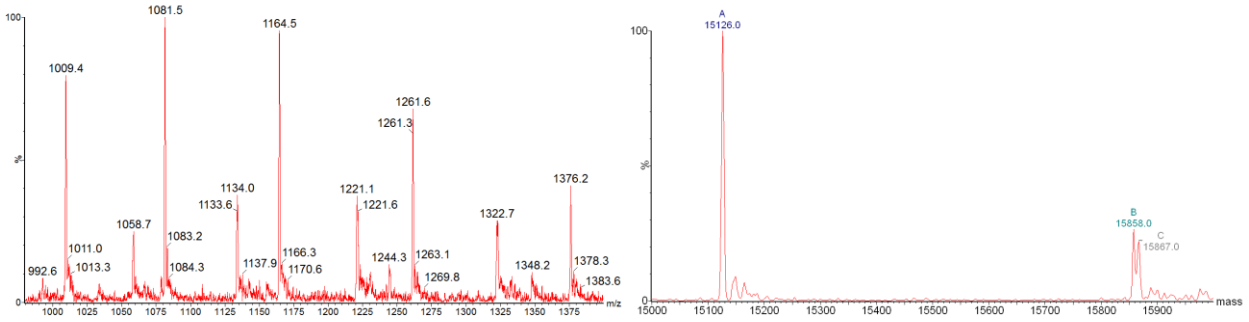
Progenesis QI Software for Metabolome and Lipidome Analyses

The Core has one computer workstation with Progenesis QI software installed locally, with another network license due to come on board in November. This software is designed to aid untargeted analysis of LC/MS data generated in metabolome and lipidome analyses by automatically detecting and integrating peaks, performing retention time alignment, coordinating searches of spectrum databases for metabolite identification, generating principal component analysis, clustering metabolites based on abundance correlations, and exporting results for further statistical analysis (*e.g.* to software such as Umetrics EZ-Info. Contact us at MassSpec.rtsf@cns.msu.edu to learn more.



Tips and Techniques. In each newsletter we intend to provide information about a selected technique available at the Core that has potential utility for the MSU research community. For this newsletter we choose to highlight a fast and accurate mass spec based method for determining protein molecular weights. Anyone who is expressing a protein in a heterologous system (for enzyme assay testing, antibody production, or any other downstream application)

would benefit from knowing whether the protein they have 'purified' is indeed what they expect it to be. It is not uncommon for small truncations or other modifications (e.g. addition of β-mercaptoethanol through disulfide bridges) to occur that are not evident using SDS-PAGE analysis but can affect protein functions. Obtaining a mass spectrum of a protein sample followed by use of software for deconvolution of the spectra can give an accurate mass of the protein sample being analyzed. A typical analysis would involve injection of 5-10 µl of a protein solution (with a concentration in the range of 1-10 µM) onto a short desalting column. Salts are not retained and wash away, then the protein is eluted within about a minute and ionized using positive-ion mode electrospray ionization (ESI). The ESI mass spectrum of a protein usually consists of multiple charge states, but this information can be deconvoluted to calculate the mass of the neutral protein using the MaxEnt (Maximum Entropy) program available in the MSMC as part of the Waters Masslynx software package. Protein masses are usually accurate to ±0.01% using ESI. An example is shown here for a sample of hemoglobin that consists of α and β chains. This particular sample also contains the Hafnia variant of the β chain. The positive-ion mode spectrum (*m/z* 980-1400; left panel) shown here illustrates several of the different charge states that are detected in the mass spectrometer. Using the MaxEnt 1 tool set for an expected mass range of 14,800 to 17,000 Da, the software accurately determines the molecular weight of the α chain at 15,126 Da (right panel), the normal β chain of 15,867 Da, and the Hafnia variant that harbors a His→Gln change resulting in a mass of 9 Da less at 15,858 Da. Similar analyses were



recently used by Dr. Bob Hausinger's group (MMG/BMB) in a study that identified a nickel containing prosthetic group covalently attached to a lactate racemase (published in the July 3, 2015 issue of Science). The MSMC has protein desalting columns and accepts samples for submission. Alternatively, researchers can be trained to perform this analysis themselves. A single sample submitted for analysis by the Core staff only costs $26.50. Alternatively, trained QTof users can get an accurate molecular weight for $11.75/sample. Contact us at MassSpec.rtsf@cns.msu.edu for additional information. For other protein identification such as analysis of digested protein samples, users should still contact the RTSF Proteomics Core.

Recent publications from Core users

Chodavarapu S, Jones AD, Feig M, Kaguni JM (2015) DnaC traps DnaB as an open ring and remodels the domain that binds primase. Nucleic Acids Res DOI:10.1093/nar/gkv961

Desguin B, Zhang T, Soumillion P, Hols P, Hu J, Hausinger RP (2015) METALLOPROTEINS. A tethered niacin-derived pincer complex with a nickel-carbon bond in lactate racemase. Science **349**: 66–9

Ekanayaka EAP, Celiz MD, Jones AD (2015) Relative mass defect filtering of mass spectra: a path to discovery of plant specialized metabolites. Plant Physiol **167**: 1221–1232

Ghosh B, Jones AD (2015) Dependence of negative-mode electrospray ionization response factors on mobile phase composition and molecular structure for newly-authenticated neutral acylsucrose

metabolites. Analyst **140**: 6522–6531

Li K, Buchinger TJ, Bussy U, Fissette SD, Johnson NS, Li W (2015) Quantification of 15 bile acids in lake charr feces by ultra-high performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci **1001**: 27–34

Liu J, Rice A, McGlew K, Shaw V, Park H, Clemente T, Pollard M, Ohlrogge J, Durrett TP (2015) Metabolic engineering of oilseed crops to produce high levels of novel acetyl glyceride oils with reduced viscosity, freezing point and calorific value. Plant Biotechnol J **13**: 858–65

Luo H, DuBois B, Sgambelluri RM, Angelos ER, Li X, Holmes D, Walton JD (2015) Production of (15)N-labeled α-amanitin in Galerina marginata. Toxicon **103**: 60–4

Mavangira V, Gandy JC, Zhang C, Ryman VE, Jones AD, Sordillo LM (2015) Polyunsaturated fatty acids influence differential biosynthesis of oxylipids and other lipid mediators during bovine coliform mastitis. J Dairy Sci **98**: 6202–6215

Ning J, Moghe G, Leong B, Kim J, Ofner I, Wang Z, Adams C, Jones AD, Zamir D, Last RL (2015) A feedback insensitive isopropylmalate synthase affects acylsugar composition in cultivated and wild tomato. Plant Physiol pp.15.00474–

Pollard M, Delamarter D, Martin TM, Shachar-Hill Y (2015a) Lipid labeling from acetate or glycerol in cultured embryos of Camelina sativa seeds: A tale of two substrates. Phytochemistry **118**: 192–203

Pollard M, Martin TM, Shachar-Hill Y (2015b) Lipid analysis of developing Camelina sativa seeds and cultured embryos. Phytochemistry **118**: 23–32

Schilmiller AL, Moghe GD, Fan P, Ghosh B, Ning J, Jones AD, Last RL (2015) Functionally Divergent Alleles and Duplicated Loci Encoding an Acyltransferase Contribute to Acylsugar Metabolite Diversity in Solanum Trichomes. Plant Cell **27**: 1002–1017

Soltanzadeh B, Jaganathan A, Staples RJ, Borhan B (2015) Highly stereoselective intermolecular haloetherification and haloesterification of allyl amides. Angew Chem Intl Ed **54**: 9517-9522.

Sungsuwan S, Yin CJ, Huang X (2015) Lipopeptide-coated iron oxide nanoparticles as potential glycoconjugate-based synthetic anticancer vaccines. ACS Appl Mater Inter **7**: 17535-17544.

Thornburg CK, Wortas-Strom S, Nosrati M, Geiger JH, Walker KD (2015) Kinetically and crystallographically guided mutations of a benzoate CoA Ligase (BadA) elucidate mechanism and expand substrate permissivity. Biochemistry **54**: 6230-6242.