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Investigating the effectiveness of HILIC-type column chemistries for oligonucleotides

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Analyzing DNA and RNA has become increasingly important as more is learned about the transcriptome and more RNA-based drugs are being produced in an attempt combat various diseases. Traditionally, liquid chromatography – mass spectrometry (LC-MS) analysis of nucleic acids is done by digestion of the sample into nucleotides and oligonucleotides and then building the sequence from the bottom-up. The gold standard for oligonucleotide chromatography has been ion pairing reverse phase liquid chromatography (IP-RP-LC). IP-RP-LC utilizes mobile phase additives that aid the chromatographic retention and gas-phase ionization of oligonucleotides. While this method has been very effective, ion pairing reagents are inconvenient to work with and difficult to eliminate from HPLC instruments. Additionally, ion-pairing reagents can lead to increased background noise in mass spectrometry when trying to change assays or modes. Thus, most oligonucleotide assays can require dedicated instruments, which is not practical and can be very costly. Recently, hydrophilic interaction liquid chromatography (HILIC) for oligonucleotide separation during LC-MS analysis has been shown to provide competitive chromatographic and mass spectrometric figures of merit. Currently, assays have been reported for a diol and amide functionalized column. The focus of this project is to further understand the retention mechanism as well as the limitations of HILIC-type columns for oligonucleotide LC-MS assays.

Dereplication and Isolation of Secondary Metabolites of the Two Selected U.S. Coastal Lichens *Mobergia calculiformis* and *Niebla josecuervoi*

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Lichens are the result of the symbiotic partnerships between fungi and macroalgae or cyanobacteria. This association leads to the production of secondary metabolites that are specific to these symbionts and are produced mostly by the fungal partner. Some of these metabolites can constitute up to 30% of the dry mass of the lichen. For example, in the current study, the well-characterized despidones norstictic acid (from *Mobergia calculiformis*) and salazinic acid (from *Niebla josecuervoi*), identified primarily by ^{1}H-NMR and LC-HRESIMS, comprised roughly 1.6% and 1.0%, respectively, of the dry mass of the lichens. Unfortunately, the presence of a single major metabolite presents a challenge in identifying and isolating minor compounds that can be new and pharmacologically interesting. Thus, the use of fractionation methods along with the integration of sensitive detection methods such as LC-MS will help in the early dereplication of known metabolites. Consequently, in the current study, LC-MS^2 and NMR, especially 1D selective methods such as 1D-TOCSY and 1D-NOESY, were used to initially identify compounds and guide the isolation process. Data obtained from the LC-MS^2 acquisition will be further analyzed by Feature-Based Molecular Networking (FBMN), from the Global Natural Products Social Molecular Networking (GNPS) platform, in order to assess the similarity of the secondary metabolites present. The FBMN uses MS^2 spectra in conjunction with compound "features" such as *m/z* and retention time in order to compare their spectral and thus structural similarity. Hence, the molecular networks obtained will not only help in annotating compounds and analyzing chemical diversity but also in visualizing the similarities of the two lichen species in terms of their chemistry. The results of this molecular networking analysis will be described in the poster presentation.

Accelerating DIA studies with fast microflow LC and Zeno SWATH acquisition

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The Zeno trap technology provides significant gains in peptide fragment signal by trapping ions in the Zeno trap region of the collision cell, then releasing them such that all ions arrive as a condensed packet at the same time in the TOF accelerator region. Targeted quantification using Zeno MS/MS was performed to measure sensitivity impact for peptide workflows. A targeted MRMHR assay was developed on 804 heavy labeled peptides using a 20 min microflow gradient. Calibration curves were generated in plasma with and without Zeno trap activation. On average, MS/MS fragment peak area increased ~5.6 fold, and the median LLOQ on column in matrix was 193 amol.

Next, data independent acquisition workflows using Zeno MS/MS were investigated to understand how this fragment ion sensitivity gain would impact DIA workflows. Key parameters for high-quality DIA data are the number of Q1 windows (which affects S/N) and MS/MS accumulation time (which impacts MS/MS sensitivity). These parameters set the cycle time, which must be optimized for each LC gradient.

Starting with the 45-min gradient, parameters were varied and the impact on the number of proteins quantified was assessed. The optimal cycle time was ~1.9 s. Several loads of K562 were analyzed with the optimized method. Data was processed using two software pipelines and slightly different method parameters worked best for the different software pipelines. The activation of the Zeno trap resulted in over 80% gains in proteins identified at a 200 ng load with a FDR of <1% and a %CV of <20.

A similar optimization was performed for a 20-min gradient, resulting in an optimal cycle time of 1.1 seconds. The full optimization results are presented, including results for 5- and 10-min gradients with full load curves from a few ng to hundreds of ng to fully characterize this new technique.

Ligand-Binding Interactions of Transthyretin with Surface-Induced Dissociation

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Transthyretin, a tetrameric protein associated with amyloid disease, and its ligand binding interactions have been studied extensively to find binding partners to stabilize the tetrameric structure of the complex to prevent the dissociation and subsequent aggregation of the monomers. The dissociation of TTR into its dimer then to monomeric subunits and subsequent aggregation of the misfolded monomers cause TTR amyloidosis which can lead to cardiomyopathy. The strengths of the binding interactions of small molecules to the tetrameric complex play a key role in the stabilization of the overall complex. Surface-induced dissociation (SID) can play a role in identifying the relative strengths of these interactions in the gas-phase. SID, an ion activation technique in the gas phase, has been shown to produce native-like fragments of protein complex subunits with the ability to retain ligand binding interactions. The SID products of tetrameric TTR provide information about the type of binding interactions. The binding partners studied are L-thyroxine (T4), the natural ligand binding partner, resveratrol, a natural product, and tafamidis, the FDA approved preventative drug for TTR amyloidosis, and ligands provided by Sanofi. T4, which binds through primarily hydrophobic forces, is not shown to stay on subunits after fragmentation. Resveratrol with hydrogen bonding and tafamidis with electrostatic interactions both stay on the subunits to a certain degree after SID. Fragmentation is performed across a set of fragmentation voltages to create energy-resolved mass spectrum (ERMS) plot, while also comparing intensities of the ligand-bound peaks at a specific voltage. Measuring the retention of small molecules on subunits in the gas-phase will increase the capabilities of the measurements SID can provide. These measurements can also be used to develop a high-throughput mass spectrometry-based method for screening future target small molecule binding partners.

Creating an LC-MS/MS method for discovering and characterizing photo-oxidative degradation products of modified nucleosides in tRNA

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Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is a proven method for the identification and characterization of post-transcriptionally modified nucleosides. Because ribonucleosides fragment during collision-induced dissociation at the N-glycosidic bond between the nucleobase and the ribose group, neutral loss scans (NLS) are commonly used during nucleoside analysis. Previously, our lab began exploring the impacts of oxidative damage on post-transcriptional modifications. By exposing samples to UVA radiation, we found that many modified nucleosides are readily degraded. However, we were not readily able to determine what the photodegradation products were. In this work, we have developed an NLS approach that allows for the discovery of photodegradation products. When combined with high mass accuracy LC-MS/MS, spectral matching, and stable isotope labelling (SIL), we are now able to both detect and characterize the end products arising when post-transcriptional modifications are damaged by photo-oxidation. These findings reveal that stable photoproducts are present on intact tRNAs. Future work will focus on understanding the biological significance of these photoproducts formed upon UVA exposure.

Key words: NLS, LC-MS/MS, SIL, spectral matching, post-transcriptional modifications, tRNA, UVA rays

A Multidimensional Approach to Probing the Binding Affinities of Polylysine and Polystyrene Sulfonate Polyelectrolytes Complexes

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Polyelectrolytes are polymers whose repeat units contain ionizable functional groups. Similar to traditional electrolytes, they can dissociate into aqueous solutions, releasing their counterions and allowing them to bear one or more charges depending on the solution. Most importantly, their ability to form complexes with oppositely charged molecules via electrostatic interactions has led to their widespread use in a number of different fields. For example, polyelectrolyte multilayer (PEM) nanofilms have become a commonplace in drug delivery applications due to their polymeric conformations being easily modified. For this reason, understanding the relative binding strengths of these complexes is crucial for these applications as well as providing useful information on how these complexes change based on the number of repeat units in each polyelectrolyte. Mass spectrometry (MS) allows for the identification of these complexes with high sensitivity and accuracy. In this study, energy-resolved mass spectrometry (ER-MS) is used to gain insight on the stability of poly-L-lysine (PLL), polystyrene sulfonate (PSS) and their polyelectrolyte complexes. PLL is a natural cationic poly(amino acid) used in tissue cultures and drug delivery. PSS is a common polyanion used as a superplasticizer in industrial materials as well as in biomedical engineering. The negatively charged sulfonate group in PSS allows for an accessible binding site for the negatively charged PLL amine group. In ER-MS experiments, the collision energy is varied (10-70 eV), providing a two-dimensional spectrum resembling a breakdown curve. This is in contrast to a traditional single dimensional spectrum given by collision-activated dissociation (CAD) at a fixed energy. A Waters Synapt G1 equipped with electrospray ionization and traveling wave ion mobility was used to ionize and separate complex ions prior to CAD with Ar(gas). Using these data, we were able to differentiate the binding strengths of the different complexes these polyelectrolytes form.

A quantitative exploration of wounding-induced changes to tomato steroidal glycoalkaloid profiles in diverse tomato fruits

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It is well documented that mechanical wounding has the capability to produce a variety of local and systemic effects in plants, including alterations to their phytochemical profiles. Tomato steroidal glycoalkaloids (tSGAs) are a class of insecticidal and fungicidal cholesterol-derived metabolites produced by members of the tomato clade. Mass spectrometry-based imaging techniques such as MALDI-MSI have elucidated qualitative changes in metabolite composition at the site of wounding in tomato fruit; however, these results lack quantitative details. Additionally, these studies have evaluated post-harvest fruit from a limited range of cultivars over relatively short periods of time. To contribute quantitative analysis of wounding-induced phytochemical profile changes on a whole-fruit scale, factor in on-plant maturation, and examine the responses across cultivars, this study examined the tSGA profiles of wounded tomato fruits across four accessions via ultra-high performance liquid chromatography, tandem mass spectrometry (LC-MS/MS). Four diverse accessions of tomato were selected –Tainan, LA2522, OH8243, and LA2213–due to their differing levels of baseline alkaloid content and varied degrees of cultivation. Fruits were wounded on-plant at the mature green and mature red maturation stages to provide insight into the endurance and intensity of any potential changes to tSGA profiles. Overall, this study aims to explore the inducibility of tomato steroidal glycoalkaloid profile changes and further illuminate tomato wounding responses throughout the ripening process and across diverse accessions.

Pharmacokinetics of Tomato Steroidal Alkaloids in Healthy Adults Following Consumption of Two Different Tomato Juices

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Steroidal alkaloids derived from tomatoes are gaining traction as a potentially health beneficial class of phytochemicals based on a growing number of *in vitro* and *in vivo* studies. Prior to investigating the bioactivity of tomato steroidal alkaloids (TSAs) in humans, understanding their pharmacokinetic behavior after absorption is important. The objective of this study is to elucidate the pharmacokinetics, bioavailability, and metabolism of TSAs following a single tomato juice containing meal. Healthy subjects (n = 11, 6M/5F) participated in a randomized crossover trial where they consumed 94 g juice (low dose) and 505 g juice (high dose) with a two-week washout period in between doses. Blood samples were collected at 11 time points over 12-hours following test meal consumption, and plasma was isolated to be analyzed using UHPLC-QTOF-MS. Alpha-tomatine and tomatidine, two of the most studied TSAs in literature and those with existing authentic standards, were found to be present in plasma from both the low and high doses indicating absorption. Bioavailability for alpha-tomatine was estimated using baseline-corrected area under the curve (AUC) values (low dose: 0.067 +/- 0.014 (nmol·h)/L, high dose: 0.195 +/- 0.065 (nmol·h)/L). The concentration over time curve for tomatidine revealed three distinct peaks within the 12-hour collection period potentially indicating multiple metabolic events of glycosylated TSAs that contribute to the observed absorption pattern. MS/MS fragmentation was used to annotate additional analytes where authentic standards are not available, revealing the presence of hydroxylated and sulfonated metabolites not endogenously present in tomato suggesting phase I and phase II metabolism. This study reports the first pharmacokinetic data for TSAs which agree with previous observations of their presence in biological fluid and provides valuable data to understand their metabolic fate after consumption of a tomato containing meal.

Characterizing Protein Complexes Using Top-Down Electron Capture Dissociation Coupled with Ion Mobility MS

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Native mass spectrometry (nMS) enables non-covalent interactions to be retained in the gas phase and has become a powerful tool in studying the structure of proteins and protein complexes. Here we describe the combination of electron capture dissociation (ECD) and ion mobility (IM) to study the structure of native protein complexes. ECD is a fast and efficient ion activation method that is installed post mobility; this allows for the application of IM to separate protein complexes by their different conformations and then fragment them by ECD. As an electron-based dissociation method, ECD can sometimes cause protein backbone cleavage while retaining non-covalent interactions, which has proven invaluable in native top-down proteomics studies. ECD fragmentation patterns will reveal protein 3D structure information. Current ECD results for protein complexes show that ECD fragmentation occurs primarily in the surface exposed regions. IM-MS has also enabled the acquisition of both native protein structures and unfolding pathways via use of collision-induced unfolding (CIU) and surface-induced unfolding (SIU) experiments. The conformational changes from CIU/SIU can be detected by drift time from ion mobility. Different experiments such as CIU-ECD-CID and SIU-ECD-CID allow ECD to result in increased sequence coverage as well as an increased understanding of protein unfolding pathways when combined with pre-IM activation experiments (CIU/SIU). Current ECD results for C-reactive protein (CRP) and transthyretin (TTR) protein standards show that the different conformations generated by CIU/SIU result in different ECD fragmentations. These data reveal both structural information and the unfolding pathway. Another protein complex, FraB, which is a deglycase converting 6-phosphofructose-aspartate to L-aspartate and glucose-6-phosphoate, is also tested to study different conformational structures by using IM-ECD. Our plans include the characterization of FraB mutants by ECD. We will also test protein complexes with a wide range of oligomeric states and molecular weights.

Identification and Mitigation of an Interferent Causing an Overestimation of Acrylamide in Food

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In regard to the Nestle analytical method for the determination of acrylamide in food, which utilizes an extraction procedure described in EN 16618:2015, an issue was identified where values of acrylamide were not matching historical data due to inconsistent peak shape. Subsequent investigation using a different column allowed for partial resolution of an unknown co-eluting compound of the same mass transitions causing potential overestimation of the acrylamide values. Identification of the compound of interest was necessary to determine if its contribution was to be excluded or not. Support from Nestle Research was able to identify the compound as N-acetyl-β-alanine by liquid chromatography high resolution mass spectrometry (Desmarchelier A, Hamel J, Delatour T. 2020. Sources of overestimation in the analysis of acrylamide-in coffee by liquid chromatography-mass spectrometry. *J Chromatogr A.* 1610:460566. doi:10.1016/j.chroma.2019.460566.) Once identified, method parameters were developed and tested by participating laboratories including Nestle Research, PTC Orbe, and NQAC Dublin to fully resolve the interferent. Method modifications requiring minimal impact to the laboratories performing routine analysis were then conducted in a cross-laboratory validation. Additional information on the work performed by Nestle Research and participating Nestle Quality laboratories regarding the identification of the interferent and subsequent method modifications is described in the publication: Aurélien Desmarchelier, Aude Bebius, Frédérique Reding, Ashley Griffin, Marta Ahijado Fernandez, Jason Beasley, Emilie Clauzier & Thierry Delatour (2022): Towards a consensus LC-MS/MS method for the determination of acrylamide in food that prevents overestimation due to interferences, *Food Additives & Contaminants: Part A*, DOI: 10.1080/19440049.2021.2022773.

Integrating metabolomics and quantitative analysis to examine metabolic differences between near-isogenic high-alkaloid tomatoes

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The tomato clade synthesizes a unique class of specialized metabolites called steroidal glycoalkaloids (tSGAs). Several studies suggest tSGAs may benefit human health. The goal of this work is to develop nearly isogenic tomatoes with high and low levels of a specific tSGA, alpha-tomatine, to enable testing of nutritional hypotheses about tSGAs in the context of tomato-containing diets. However, one report suggests alpha-tomatine may impart an off-taste to the fruit. We aim to determine how nearly isogenic high alpha-tomatine tomatoes differ metabolically, and whether alpha-tomatine enhancement has any negative flavor/liking implications. We crossed a wild, bitter, high alpha-tomatine cherry-type tomato (LA2213) with a processing-type tomato (OH8243, low alpha-tomatine) to generate progeny which genetically segregate for high-alpha tomatine. Our strategy used an inbred-backcross breeding scheme and DNA-based genetic markers to identify progeny that are nearly identical to OH8243 except for increased alpha-tomatine. Breeding to increase a single metabolite may have unintended consequences due to genetic phenomena like gene families, linkage drag, and pleiotropy. We addressed these pitfalls by targeted quantification of 18 tSGAs using LC-MS/MS, and assessing the metabolome using LC-QTOF-MS. We evaluated selections from a OH8243×LA2213 advanced progeny and assessed subsequent self-pollinated generations. Additionally, three bitter and high alpha-tomatine wild cherry-type tomatoes, a domesticated cherry-type tomato, and OH8243 were grown in triplicate in two locations in a randomized complete block design. All plots (n = 39) underwent assessment for tSGAs and global metabolic profiling (methanolic extraction, positive and negative polarities). Principal components analysis was used to assess similarity of OH8243×LA2213 advanced progeny to OH8243 and bitter tomatoes. Using univariate and multivariate approaches, we plan to elucidate features differing between near-isogenic high-alpha tomatine tomatoes. These features will then be identified, isolated, and integrated with future sensory studies.

A versatile pseudo-MS^3 approach for pinpointing the C=C bond in phospholipids

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Mass spectrometry (MS), generally coupled to liquid chromatography (LC), is a powerful tool for qualitative and quantitative lipid analysis. High-resolution mass analysis of molecular ions followed by the analysis of the fragment ions after collision induced dissociation (CID) can provide not only the exact mass of the analyte but also its structural information. Nevertheless, elucidating the complete structures of lipids with MS is still limited. For example, the identification of the C=C bond location in fatty acyl chains is still challenging with commercial mass spectrometers. Low-energy CID of protonated or deprotonated lipids seldom yields specific fragment ions indicative of the C=C position. Here, we present a pseudo MS^3 method, employing in-*source* fragmentation (ISF) followed by CID, to identify the position of the C=C bond. Epoxy-phospholipids, produced by reaction with m-CPBA (meta-chloroperoxybenzoic acid), are introduced into the ionization source where in-source fragmentation is activated. The epoxy-fatty acyl group, generated by ISF, undergoes CID and produces diagnostic ion pairs that are 16 Da apart. Because this method does not require a specific mass analyzer for MS^3 or any instrument modification, it can be used in any tandem mass spectrometer. To validate the versatility of this pseudo-MS^3 approach, we performed ISF and CID in three different mass spectrometers, with CID performed in the linear ion trap of a Thermo Orbitrap XL mass spectrometer, in the HCD cell of the Thermo Q-Exactive Plus, and in the collision cell of the Agilent Q-TOF 6545. Work to date was performed by infusion, with the pseudo MS^3 approach showing better sensitivity than the conventional MS^3 method. However, as we are not able to differentiate the fragments of co-eluting analytes with the pseudo MS^3 method, separation techniques like LC are required. In continuing work, we will apply LC prior to the pseudo MS^3 method.

Electron Capture Charge Reduction Assists in Native Mass Spectrometry of Intact Nanodiscs

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Membrane proteins govern many key cellular processes including transport across the membrane, intercellular communication, and cell-cell adhesion. Understanding membrane protein structure and function is important for informing drug design and disease treatment. Structural characterization of membrane proteins by classical techniques has been difficult due to their hydrophobicity, flexibility, and lack of stability. However, native mass spectrometry (MS), which retains noncovalent interactions in the gas phase, has been used in the past to reveal an abundance of structural, functional, and mechanistic information from membrane proteins and membrane protein complexes. For native MS studies, membrane proteins have typically been solubilized in detergent micelles, which are simple to assemble and keep membrane proteins from aggregating, while at the price of difficult removal of the native protein. Nanodiscs and liposomes are two other membrane mimetics that are becoming more popular in native mass spectrometry, mainly for their ability to retain the native-like environment in the form of specific lipid bilayer interactions that have been found to be important to structure stability and function. The goal of this project is to characterize membrane proteins and intact nanodiscs by native mass spectrometry on the Thermo QE UHMR Orbitrap MS with a modified electron capture dissociation (ECD) cell. Unloaded, intact nanodiscs are difficult to resolve using common native MS practices due to their polydispersity. Gas-phase charge reduction by way of ECD will be used to resolve these polydisperse samples. Antimicrobial peptides, which are known to incorporate and oligomerize only in the presence of a lipid bilayer, will be used to confirm properly resolved intact nanodiscs by gas phase charge reduction.

Petroleomic Aromatic Acid Dissociation Chemistry

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Aromatic carboxylic acids and classical naphthenic acids are a major cause of the naphthenic acid corrosion problem experienced by petroleum industries. Unfortunately, the dynamics of the naphthenic acid corrosion mechanism have not been fully understood due to limitations in petroleum characterization. Fortunately, the recent development of ultrahigh-resolution mass spectrometry has provided a better understanding of both the broad structural and elemental composition of individual petroleum compounds and isomers. Some historical techniques used for crude oil characterization such as total acid number (TAN) have been used to indicate petroleum acidity. This titration method unfortunately does not quantify the amount of carboxylic acids present, and its results are not proportional to the corrosiveness of a sample, as it does not account for the influence of structure on acidity. Furthermore, not much research has been done on petroleum isomer differentiation. Differentiating isomers of petroleum acids is an important step in understanding acid reactivity and corrosivity potential of naphthenic acids in petroleum crude oils. In this research, we use a combination of tandem mass spectrometry and computational chemistry as a means for isomer differentiation and an understanding of the dissociation chemistry that makes each isomer unique and allows their unique features to aid in differentiation. Electrospray Ionization tandem mass spectrometric experiments were performed in a Q-Exactive Orbitrap. Systematically varied collision-induced dissociation of deprotonated precursor ions enabled characterization of the fragmentation pathways and their energy dependence. Supporting computational (Gaussian-16) studies of the gas phase ion structures and competing dissociation pathways with multiple levels of theory beginning with b3lyp /631+G(d,p) for multiple minima, transition state (TS) were performed. Intrinsic reaction coordinate calculations identified specific structures linked by each TS. This structural information contributes to the mass spectral library and provides fundamental concepts that aid naphthenic acid characterization on both a research and an industrial scale.

Native Mass Spectrometry of Zinc Binding by Txn Repressor Loz1

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Zinc is an essential cofactor for many biological systems but can be toxic in excess. Therefore, intracellular Zn concentrations are under tight control in *Saccharomyces pombe.* Loss of Zinc 1 (LOZ1) is a 55kDa protein with two t Zn finger domains, necessary for zinc regulation in *S. pombe.* The double zinc finger domain and accessory domain alone have been shown to be the smallest truncation of the protein that is still active. Zinc binding to Loz1 is thought to regulate its binding to LRE DNA sequences, thereby leading to transcriptional repression. Here we explore the use of nMS to characterize the thermodynamics of zinc binding to Loz1 and Ion Mobility MS to characterize the accompanying structural changes in the protein.

RAD52-DBD Binding with DNA by Native MS and Mass Photometry

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DNA double strand breaks (DSB) can result in chromosomal aberration, which causes the development of various cancers. Homologous recombination (HR) is an essential process for repairing DSB and radiation sensitive 52 (RAD52) is one of the most important proteins that is involved in this process. For DNA repair, RAD52 binds with two complementary ssDNA, but structures of different Rad52-DNA intermediates and the chemical kinetics for forming the RAD52-DNA complex are still unclear. Native mass spectrometry (MS) retains noncovalent interactions in the gas-phase to characterize native-like protein-DNA complexes, and surface induced dissociation (SID) is a gas-phase activation method that can provide information for the connection between different protein subunits, which is useful for characterizing oligomeric protein complexes. Also, Mass Photometry (MP) is a solution phase technique that measures masses of single molecules/complexes directly by light scattering. Starting with the DNA binding Domain (DBD) of RAD52, we used native MS combined with SID to acquire structural information of RAD52 and its complexes with DNA. Additionally, MP was used to provide the complementarity of native MS . These results will provide better insights on DNA repair, which will lead to further therapeutic development to treat various cancers.

Development of a publicly available LC-MS/MS spectral library for the analysis of the *Rosaceae* metabolome

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Metabolite identification is one of the most challenging parts of plant metabolomics work. To conclusively identify a metabolite, an authentic standard is needed, which is a challenging requirement because 1) many phytochemicals are not available for commercial purchase, and 2) these materials can be very expensive. It is possible to search MS^2 data against publicly available spectral databases like GNPS and HMDB though this often yields few matches and does not typically take into consideration chromatographic information. Our goal is to create a standard chromatography method and publicly available MS^2 library for the analysis of the apple metabolome. For this work, we accounted for seventy authentic standards containing phytochemicals previously reported in *Malus spp.,* such as flavonoids, flavanols, dihydrochalcones, and sugars. Raw data was collected on an Agilent 1290 LC interfaced with a 6545 HRMS Q-TOF. Authentic standards and quality control samples were subjected to MS^1 scan as well as MS^2 product ion experiments in positive and negative polarity. For the MS^2 experiments, samples were run at 20 and 40 eV to capture the fragmentation pattern of the standards under different conditions. Collected data will be deconvoluted using MZmine, and include retention time, MS1, and MS2 data, along with metadata such as monoisotopic mass, molecular formula, InChIKey, CAS number, ChemSpider ID, KEGG ID, among others. The spectral library will be made publicly available in a standardized format (mgf) for future use and modification. With the creation of this resource, we expect to facilitate metabolite identification by providing a high-quality MS^2 library for the *Rosaceae* family, providing the ability for a level 1 identification with retention time and MS2 fragmentation data.

Universal Mass Exclusion List for Enhanced Modification Mapping of RNA

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Determining the locations of modified nucleosides within RNA sequences is challenging, yet important to fully understand how such modifications impact the biological roles of RNAs. We have developed a number of methods for modification mapping of RNA using liquid chromatography-tandem mass spectrometry (LC-MS/MS) approaches. Many of these approaches require the enzymatic digestion of RNA into constituent smaller oligonucleotides that can be separated and analyzed by LC-MS/MS. A major challenge during modification mapping by LC-MS/MS is the presence of unmodified oligonucleotides (unmodified digestion products), as these can be more abundant than or co-elute with modified digestion products. Previously we demonstrated that excluding the mass values of unmodified oligonucleotides during data acquisition can improve the detection of modified digestion products. As all RNAs are made from four canonical nucleotides (A, U, C, and G), combinations of these nucleotides in a defined oligonucleotide length can be computed as an exclusion list. The goal of this work is to develop a genome-independent universal mass exclusion list (UMEL) of unmodified oligonucleotides for a defined size range and test the exclusion efficiency during LC-MS/MS. Our results indicate that the UMEL in combination with hydrophilic interaction chromatography improves the detection of low abundance modified digestion products during RNA modification mapping. We will also report the applicability of an enzyme-specific UMEL for modification mapping of the tRNA population of a well-characterized model organism, *Escherichia coli*, and the poorly characterized fission yeast, *Schizosaccharomyces pombe*.

Measuring Metabolic Flux in Colon Adenomatous Organoids

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Tumorigenesis is associated with metabolic reprogramming, which presents opportunities for therapeutic exploits. Stereotypical metabolic effects include increased glucose uptake and glycolytic flux, as well as altered lipid and amino acid metabolism. While metabolic changes are better characterized in invasive cancers, there is currently limited data on metabolic reprogramming in early-stage tumors (adenomas). Tracer metabolomics studies and metabolic flux data provide powerful tools for simultaneously investigating multiple metabolic pathways *in vitro* and *in vivo*. Furthermore, 3D organoid cultures provide a method to better replicate *in vivo* conditions. Seahorse assays on 3D organoid cultures provide a basic method for bioenergetic profiling and metabolic flexibility. Performing tracer metabolomics and Seahorse assays on organoids however presents multiple technical challenges for the investigator- from separation of organoid material from matrix, to ensuring rapid metabolite extraction, and finally: data normalization for quantifying data. Our group has been developing methods for quantitative tracer metabolomics and extracellular flux using intestinal organoids. Our initial trials were employed as proof of principle to determine effects of APC tumor-suppressor loss on the metabolic needs of colon crypt-derived organoids (CCO's). Fluorescence-based normalization assays may allow the investigator to avoid interference by Matrigel, that normally confounds protein-based assays. We're developing a low-dose dual-staining approach for normalization, which will minimize disruption of metabolomics protocols. This approach has demonstrated results comparable to some commercial assays. By further developing these methods, we hope to provide a valuable toolset to study metabolic effects of diet and microenvironment on colorectal cancer initiation and progression.

LC-MS/MS-based detection of 5-methyl cytidine in *E. coli* tRNAs arising from oxidative stress

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Oxidative stress caused by endogenous and exogenous sources has been associated with a number of human diseases. One exogenous source is ultraviolet radiation (UVR). Our lab earlier found that post-transcriptional modifications in transfer ribonucleic acids (tRNAs) are damaged by the reactive oxygen species (ROS) generated by UVA. Those studies also revealed that some photoproducts were more readily identified during RNA modification mapping by liquid chromatography tandem mass spectrometry (LC-MS/MS) rather than relying solely on their detection during nucleoside analysis. Another approach for generating ROS is the Fenton reaction, whereby Fe^{2+} catalyzes the generation of hydroxyl radicals ($HO^{\cdot}$) from hydrogen peroxide (H_2O_2). Here we have been exposing *Escherichia coli (E. coli)* cells to ROS conditions generated by the Fenton reaction. Surprisingly, our preliminary nucleoside analysis results reveal that 5-methylcytidine (m^5C) is found at significantly higher levels in tRNAs upon exposure to Fenton conditions. Because m^5C has not been reported in any published *E. coli* tRNA sequences, we have focused our RNA modification mapping analyses on discovery possible sequence locations in specific tRNAs where this modification may be occurring. At present, Tyr-QUA appears to be the most likely tRNA containing this additional modification, which appears induced by the Fenton reaction. Results from our analyses along with a discussion into the possible causes for this change in modification status will be presented.

The ubiquitin–26S proteasome system and autophagy relay proteome homeostasis regulation during silique development

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Functional studies of the ubiquitin-26S proteasome system (UPS) have demonstrated that virtually all aspects of the plant's life involve UPS-mediated turnover of abnormal or short-lived proteins. However, developmental characterization of the UPS, including in seeds and fruits, remains scarce even though mutants of its several core elements are embryonically lethal. Unfortunately, early termination of embryogenesis limits the scope for characterizing the UPS activities in reproductive organs. Given both economic and societal impact of reproductive production, such studies are indispensable. Here, we systematically compared expression changes of multiple 26S proteasome subunits along with the dynamics of proteasome activity and total protein ubiquitylation in seedlings, developing siliques or embryos of *Arabidopsis thaliana*. Since autophagy plays the second largest role in maintaining proteome stability, we parallelly studied three late-limiting enzymes that are involved in autophagy flux. Our experiments unexpectedly discovered that, in opposite to the activities in seedlings, both protein and transcript levels of six selected 26S proteasome subunits gradually decline in immature siliques or embryos toward maturation while the autophagy flux rises albeit in a nutrient-rich condition. We also discovered a reciprocal turnover pathway between the proteasome and autophagy. While the autophagy flux is suppressed in seedlings by UPS-mediated degradation of its three key enzymes, transcriptional reprogramming dampens this process in siliques that in turn stimulates a bulk autophagy degradation of proteasomes. Collectively, our discovery about the developmental changes of the UPS and autophagy activities suggests that they relay the proteome homeostasis regulation in early silique and/or seed development highlighting their developmental interactions.