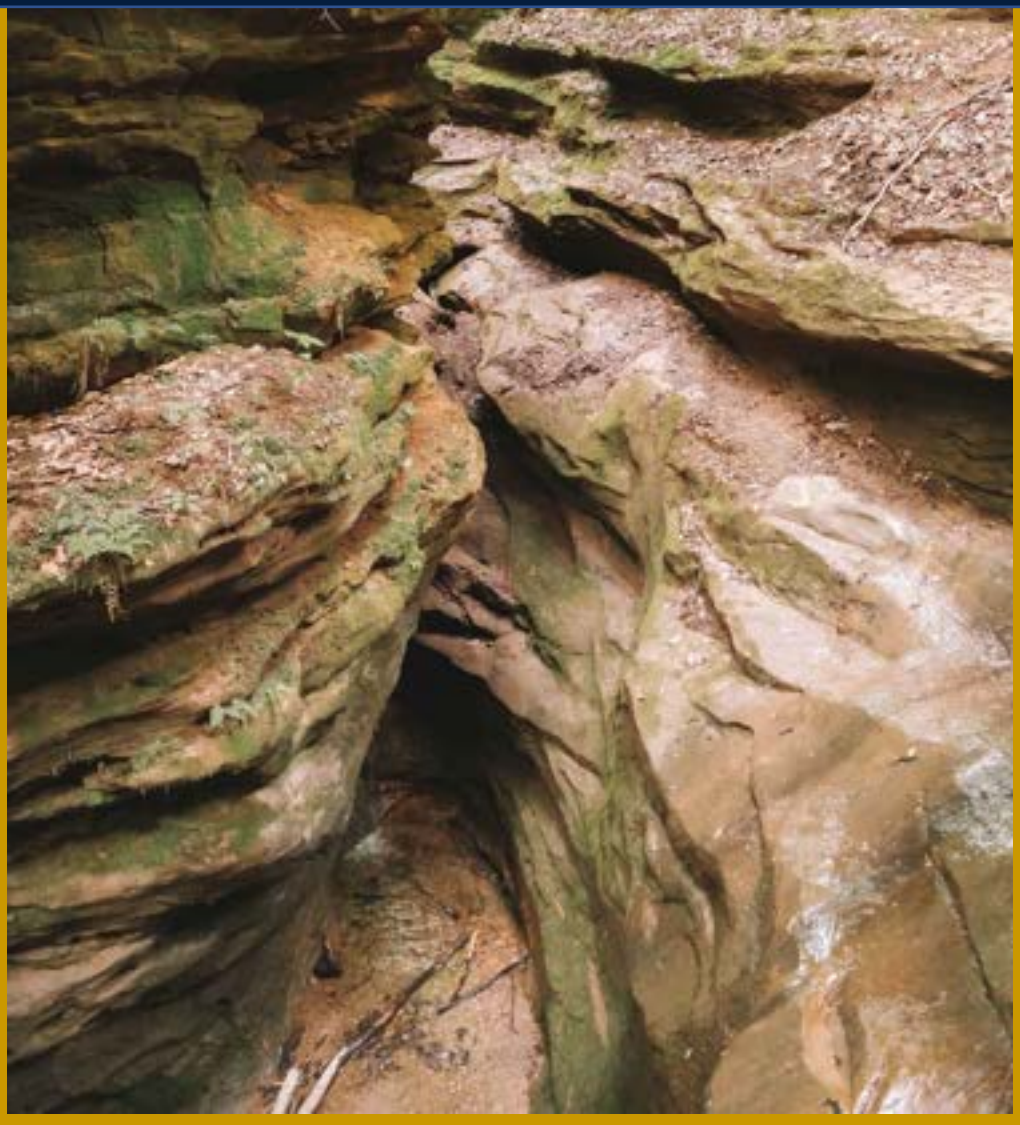
2022 TURKEY RUN ANALYTICAL CHEMISTRY CONFERENCE

Turkey Run State Park
Marshall, IN
September 30th-October 1st



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Introduction

We want to welcome everyone to the 2022 Turkey Run Analytical Chemistry Conference. Notre Dame is pleased to be hosting this year's conference in-person at Turkey Run State Park. As always, this year's conference is organized and brought to you by student representatives and includes research presentations from students from the University of Notre Dame, Purdue University, Indiana University, the University of Illinois Urbana-Champaign, and The Ohio State University.

This year's conference includes a total of 106 attendees giving 8 oral presentations and 54 poster presentations that represent a diverse body of work within analytical chemistry. Our Keynote Speaker is Dr. Marta Venier, an accomplished environmental analytical chemistry professor from Indiana University Bloomington who will be discussing her work on mass spectrometry techniques to study the fate, transport, and behavior of persistent organic pollutants in the indoor and outdoor environment.

We want to extend our gratitude to all attendees, presenters, and organizers for their efforts and support in planning this year's conference. We also thank the Turkey Run Inn for once again providing an exceptional venue for us to gather.

Conference Schedule

Friday, September 30th

3:00 – 5:00 PM	Check in
4:30 – 5:30 PM	Poster Session A- Lusk Room
5:30 – 6:30 PM	Poster Session B- Lusk Room
6:30 – 7:15 PM	Dinner Group A- Dining Room
7:15 – 8:00 PM	Dinner Group B- Dining Room
8:30 PM – 12:00 AM	Social Mixer- Tennis Shelter

**Dinner included with registration

Saturday, October 1st

7:30 – 8:30 AM	Breakfast Buffet, Eli Lilly Discussion and Q&A session- Lusk Room
8:30 – 8:40 AM	Conference Photo- Front of Inn
8:45 – 9:00 AM	Welcome- Lusk Room
9:00 – 9:50 AM	Keynote Speaker- Lusk Room
9:50 – 10:00 AM	Break
10:00 AM – 12:00 PM	Oral Presentations- Lusk Room
12:00 – 12:10 PM	Closing Remarks

**Breakfast included with registration

Keynote Speaker: Dr. Marta Venier

chemist and Assistant Professor at the Paul O'Neill School of Public and Environmental Affairs at Indiana University in Bloomington, Indiana. Dr. Venier graduated with her Ph.D. in Environmental Sciences from O'Neill School of Public and Environmental Affairs at Indiana University in 2008 under the mentorship of Dr. Ronald Hites, a leader in the field of mass spectrometry.

In her research she employs analytical chemistry and mass spectrometry techniques to study the fate, transport, and behavior of persistent organic pollutants in the indoor and outdoor environment, including both legacy and emerging pollutants. Notable works include her recent publication titled *Per- and Polyfluoroalkyl Substances in North American School Uniforms* that received international attention as well as numerous works measuring flame retardants in the Great Lakes & beyond. Since November 2019, she has been leading the Integrated Atmospheric Deposition Network (IADN), a monitoring program funded by the U.S. Environmental Protection

Agency. We thank Dr. Venier for sharing her work with us at this year's conference.

Oral Presentations

Enhanced multiply charged (EMC) scans for improved S/N ratio of doubly charged lipid species in complex extracts

Kimberly C. Fabijanczuk (1(, James W. Hager (2), and Scott McLuckey (1)
(1) Purdue University
(2) Sciex

Conventional electrospray ionization (ESI) can give rise to multiply charged analyte species that overlap in m/z space with undesired singly charged species making it difficult to analyze analytes of interest. Furthermore, if the multiply charged species are in low abundance relative to singly charged species and the sample is highly complex, identifying them proves to be even more difficult due to low signal levels and space charge effects. Cardiolipins (CLs) are structurally complex phospholipids present in low intensity in the lipidome but play crucial roles in mitochondrial metabolism and various regulatory processes. ESI of CLs in negative ion mode shows abundant doubly deprotonated ions and minor singly deprotonated ions. In lipid extracts, CLs overlap with singly charged phospholipids (e.g PG and PE) in m/z space and are in low abundance, proving it difficult to study CLs in biological extracts. To overcome this challenge, we have utilized a gas-phase separation approach to remove singly charged species from an ion-trap while maintaining multiply charged species by allowing ions to leak out in a charge state dependent fashion. Herein, we describe the considerations and applicability of applying enhanced multiply charged (EMC) scans to perform a gas-phase separation of CLs in an E. coli extract. This method allows for improved S/N of CLs with minimal overall signal loss, is performed within 300 ms, and can be done iteratively after multiple fills of the trap.

Optimizing the dimensions of in-plane circular nanopores for resistive-pulse sensing of virus capsids

Ethan D. Call (1), Tanner W. Young (1), Adam Zlotnick (1), Stephen C. Jacobson (1)
(1) Indiana University

We are developing in-plane nanofluidic devices to measure the size and surface charge of individual virus capsids. Analysis of single particles provides valuable insight into biological processes that are often missed when a population is studied as an ensemble. To characterize hepatitis B virus (HBV) capsids, we are using resistive-pulse sensing as a label-free, nondestructive technique. For particle sensing, nanopores with circular cross sections are milled into lamellae formed in nanochannels. To improve the signal-to-noise ratio, we are shortening the nanopore length by selectively thinning the center section of the lamella with a focused ion beam prior to drilling the nanopore through that section. This method provides independent control for fabricating the pore length and diameter, while maintaining sufficient surface area on top of the lamellae for bonding the cover plate. Shortening the length of the pore not only increases the signal-to-noise ratio but also improves the resolution between the size distributions of the T = 3 and T = 4 capsids, which are 31 and 35 nm in diameter, respectively. The device design, fabrication process, and results from the resistive-pulse measurements will be discussed in this presentation.

Oral Presentations

Robust Isoelectric-Mass Spectrometry Separations of Proteins

Caitlin M. Kerr (1), Olivia L. Schneider (1), Bonnie J. Huge (1), Matthew M. Champion (1)
(1) University of Notre Dame

Protein therapeutics are among the fastest growing class of drugs. Characterization of these macro-molecular compounds is essential for their advancement as candidate drugs and for their safety. Heterogeneity within these therapeutic proteins complicates this characterization and quality control. Capillary isoelectric focusing (cIEF) is a separation technique that uses amphoteric reagents to separate molecules based on their isoelectric points (pI). CIEF can separate molecules with resolutions as high as 0.005 pI units. Integrating cIEF with Mass Spectrometry (MS) detection is ideal for subsequent mass characterization. MS has the capacity to identify protein modifications that cIEF alone is unable to. One major obstacle to progress in the field is that the concentration of ampholytes required for high-resolution cIEF (>5% m/v) is mutually exclusive with robust electrospray ionization MS. Ampholytes suppress ionization and contaminate the instrument, resulting in poor sensitivity. One solution is to dilute the ampholytes; which improves ionization efficiency at the expense of sensitivity and resolution. To address this challenge, we constructed and validated a novel in-line interface between cIEF and MS. The interface consists of Nafion tubing sheathed in a stainless steel tube in which liquid counter-flow occurs. Ampholytes permeate the membrane and are discarded while the molecules of interest continue into the mass spectrometer. This interface overcomes the principle challenge of reducing the concentration of ampholytes prior to MS. We optimized our interface for amino acid ampholytes (Lys, His, Gly, Asp, Glu) using Flow Injection Analysis (FIA). It was determined that a wash concentration of 5% isopropanol alcohol was optimal for amino acid permeation. We next conducted automated high-resolution cIEF-ESI-MS. Peak shape, desalting efficiency and robustness were described. The results of this work on synthetic mixtures will be presented.

Lenses Integrated into Microfluidic Devices for Optical Trapping of Particles

Brigham Pope (1), Suhun Jo (1), Mi Zhang (1), Bogdan Dragnea (1), Stephen C. Jacobson (1)
(1) Indiana University

Integration of optical components into microfluidic devices can enhance particle manipulations, separations, and analyses. We present a method to fabricate annular nanoscale lenses within glass microchannels to precisely position optical tweezers within microfluidic devices. Integrated thin-film nanolenses perform the laser focusing required to generate sufficient particle-trapping optical forces without significant off-device beam manipulation. Moreover, the ability to trap particles with unfocused laser light allows multiple optical traps to be powered simultaneously by a single input laser. We have optically trapped and diverted polystyrene particles that are 0.5, 1, 2, and 4 μm in diameter over nanolenses fabricated in both chromium and gold thin films. Optical forces generated by these nanolenses are capable of capturing particles flowing in fluid streams up to 25 μm/s, corresponding to forces as large as 2 pN. Quantitative trapping experiments under flow conditions demonstrate size-based differential trapping of particles, matching quantitatively with theoretical values. The optical forces on these particles are identical to traditional optical traps, but the addition of drag forces and inertia from the microfluidic flow environment introduces a size selectivity that would be absent in a traditional optic tweezer setup. Thus, the combination of optic tweezers and the microfluidic platform enhance the capabilities of both individual parts.

Oral Presentations

Screening for Lead in Indiana

Ornella Joseph (1), Meghanne Tighe (1), Gabriel Filippelli (2), Marya Lieberman (1)
(1) University of Notre Dame, (2) Indiana University–Purdue University Indianapolis

Lead is a heavy metal and neurotoxin that especially affects young children, resulting in behavioral problems, speech defects and learning disabilities. Health departments require that children are tested at 12 months and 24 months. If an elevated blood lead level is detected, the child's home is required to be assessed for lead risks. This process is problematic because unless a child is lead poisoned, a home containing lead hazards will not be remediated.The Lead Innovation Team at Notre Dame developed a screening kit for lead in soil, paint, and dust in all homes. The goal in developing this kit was to identify sources of lead exposure, before a child is lead poisoned. Distribution of this kit began in 2019 and to-date nearly 600 homes in Indiana have been screened for lead. Screening for lead is important because the majority of the houses were built before lead paint was banned in 1978. Prior to the ban, lead, valued for its high opacity and quick drying qualities, made up nearly 25% of paint by weight. Paint with lead gets into the soil outside and dust inside the home, and if swallowed or inhaled, causes lead poisoning.

Single molecule spatial transcriptomic analysis of the honey bee brain

Alex Schrader (1), JuYeon Lee (2), Marisa Asadian (1), Ian Traniello (3), Gene E. Robinson (1), Hee-Sun Han (1)
(1) University of Illinois at Urbana-Champaign, 2) Millikin, 3) Princeton University

Spatial transcriptomic analysis methods have exploded in popularity in the last few years, offering valuable insights into functional neuroanatomy at the molecular level. We used sequential Fluorescence In Situ Hybridization (FISH) to study spatial gene expression patterns in the brains of the Western honey bee, Apis mellifera, which have a wide range of social behaviors orchestrated by a small and densely packed brain. Sequential FISH is a highly multiplexed method that can identify the spatial locations of hundreds to thousands of genes in a single experiment. However, cells in the bee brain are approximately three times smaller than mouse or rat neurons, making it very difficult to either use Sequential FISH or bead based spatial transcriptomic methods (such as Visium) due to the high mRNA density and densely packed cells, respectively. We have overcome these limitations by combining Sequential FISH with tissue clearing and expansion microscopy to be able to detect millions of unique transcripts in a single tissue section. We embedded cleared bee brain sections into an expandable gel matrix, and physically expanded cell volume 2X isotropically. The reduction in spot density improved spot detection rates in these small cells. Using these methods, we detected more than 3 million transcripts from 130 genes in a single 7 μm thick section. To check if gene identification was accurate, we compared the localization of dopamine receptors DopR2 and Dop3 and transcriptional activator LHX3 with results from previous FISH studies and found similar patterns. We also were able to identify spatially distinct expression patterns in the honey bee brain. For example, we identified multiple different Kenyon cell populations within the mushroom bodies, a brain region involved in learning and memory and social behavior, including those identified by classical neuroanatomical methods. We also identified subcellular patterns of gene expression in the optic lobes by comparing expression in neurites to that in somata. We plan to use this method to study spatial gene expression patterns in the brain as a function of behavioral state to better understand the molecular basis of behavior.

Oral Presentations

Molecular 3D Reference Artificial Intelligent Infrastructure for Mass Spectrometry Imaging of Microglial Cells in Mouse Brain Tissues

Connor Beveridge (1), Matthew Muhoberac (1), Kaushik Sharma (1), Palak Manchanda (1), Manxi Yang (1), Emerson Hernly (1), Lixue Jiang (1), Mushfeqa Iqfath (1), Julia Laskin (1), Gaurav Chopra (1)
(1) Purdue University

Three-dimensional (3D) molecular atlas of the brain provides biologists with powerful tools to perform in-depth molecular analysis of cell- and region-based specificity that are associated with a wide range of neurological disorders. There are many challenges with constructing these atlases, including 2D segment alignment to a 3D reference, identification of non-labeled analytes, determination of cell types, and platform generalization to a wide range of analytes simultaneously. Our approach consists of developing an artificial intelligent (AI) infrastructure to use nano-DESI mass spectrometry imaging (MSI) data that will be combined with immunofluorescence imaging to identify cell- and region-specific biologically relevant molecules in the mouse brain. Here, we used the MSI spectra of 2D tissue samples that were aligned to generate a 3D reference coordinate system using Allen Mouse Brain Reference Atlas. Next, the brain tissues stained with microglia cells, an immune cell type in the brain, were automatically segmented by training a fully convolutional neural network (FCNN) based on the Yolo architecture on full images. The reference position of MSI data is used to match analytes of interest to microglial cell positions for 3D visualization using a Python application interface. Overall, this project develops an innovative data analysis platform for the acquisition of a comprehensive spatially resolved cell-specific atlas of lipids, metabolites, and proteins in mouse brain tissue.

Automated Measurement of Electrogenerated Redox Species Degradation Using Multiplexed Interdigitated Electrode Arrays

Michael Pence (1), Oliver Rodríguez (1), Nikita Lukhanin (1), Charles M. Schroeder (1), Joaquín Rodríguez- López (1)
(1) University of Illinois at Urbana-Champaign

Characterizing the decomposition of electrogenerated species in solution is essential for applications involving electrosynthesis, homogenous electrocatalysis, and energy storage with redox flow batteries. In this work, we present an automated, multiplexed, and highly robust platform for determining the rate constant of chemical reaction steps following electron transfer, known as the electrochemical (EC) mechanism. We developed a generation-collection methodology based on microfabricated interdigitated electrode arrays (IDAs) with variable gap widths on a single device. Using a combination of finite-element simulations and statistical analysis of experimental data, our results show that the natural logarithm of collection efficiency is linear with respect to gap width, and this quantitative analysis is used to determine the decomposition rate constant of the electrogenerated species (kc). The integrated IDA method is used in a series of experiments to measure kc values between ~0.01 to 100 s-1 in aqueous and nonaqueous solvents and at concentrations as high as 0.5 M of the redox-active species, conditions that are challenging to address using standard methods based on conventional macroelectrodes. The versatility of our approach allows for characterization of a wide range of reactions including intermolecular cyclization, hydrolysis, and the decomposition of candidate molecules for redox flow batteries at variable concentration and water content. Overall, this new experimental platform presents a straightforward automated method to assess the degradation of redox species in solution with sufficient flexibility to enable high-throughput workflows.

Poster Presentations: Group A

Combined Machine Learning and Chemometrics of NIR spectra can quantify acetaminophen, enabling detection of substandard pharmaceuticals

Olatunde Awotunde (1), Marya Lieberman (1)
(1) University of Notre Dame

Advanced sensing technologies and chemometrics are central to improving identification of substandard and falsified pharmaceuticals in field settings. Vibrational spectroscopic techniques such as near infra-red (NIR) assess the vibrational energies of molecules in pharmaceuticals with prompt, precise, and non-destructive characteristics. Mathematical and statistical exploration of the spectra from these technologies provides characteristics information to distinguish fake pharmaceuticals from genuine ones. However, it is difficult to build comprehensive product libraries in field settings due to the large numbers of manufacturers who supply these markets, frequent unreported changes in materials sourcing and product formulation by the manufacturers, and general lack of cooperation in providing authentic samples. In this work, we demonstrate that a simple library of lab-formulated binary mixtures (an active pharmaceutical ingredient (API) and two diluents) gave good analytical predictions on branded acetaminophen drugs by discriminating substandard and falsified formulations of the API. Six chemometric and machine learning models that individually showed poor robustness for formulations outside the training set were combined for an optimized performance that integrates the respective unique strengths. Our end goal is to integrate NIR with the chemical functional group analysis performed by our already widely accepted paper analytical device; together, these technologies will be a more powerful tool for field screening of pharmaceutical and illicit drugs.

Automated MALDI sample preparation and well-by-well spotting

Sadie R. Schultz (1), Garrett C. McFadden (1), Matthew M. Champion (1)
(1) University of Notre Dame

Identifying and quantifying changes in protein abundance is central to understanding physiological processes. The major technique used for interrogating complex protein mixtures is bottom-up proteomics. Bottom-up proteomics is an approach where proteins are digested into peptides, which are then analyzed via mass spectrometry. One of the main techniques used for fast evaluation of these and other biological samples is MALDI-TOF MS. Sample analysis via MALDI-TOF MS is rapid; and many samples can be analyzed rapidly. However, spotting numerous samples on a MALDI target is time consuming and error prone when performed manually. We altered a commercial lab robot, an Andrew+, to perform bottom-up proteomics preparation and MALDI plate spotting. The robot uses traditional pipettes, making protocols transparent in that they closely mirror the steps a scientist takes. It is also fully compatible with heaters, shakers, and manifolds necessary for sample preparation. We previously adapted the most common techniques used in proteomics for automation: in-solution/S-Trap digestion, ZipTip desalting, and SPE desalting. We designed custom bridges and adapters and produced them on consumer-grade 3D printers to facilitate the rapid development of custom applications. We designed a MALDI bridge that allows the robot to deposit sample and matrix on each well of a MALDI plate for well by well analysis. In order to test our method and bridge, digests of the bovine serum albumin (BSA) protein were spotted on 192 MALDI wells with the robot using our custom bridge. The remaining 192 wells on the same MALDI plate were spotted manually. The automated and manual spotting produced 36.4% (σ=5.7) and 36.4% (σ=4.0) coverage of the BSA protein respectively. In the future, we plan on using this bridge for intact protein work in addition to peptide analysis. The adaptors and methods we have developed will make automation accessible to labs and will reduce the required time for manual sample preparation.

Poster Presentations: Group A

The air-gap PAD: A scalable fabrication method for paper microfluidics

Rachel M. Roller (1), Angela Rea (1), Marya Lieberman (1)
(1) University of Notre Dame

Microfluidic paper analytical devices (μPADs) are a promising platform for point-of-use testing, but manufacturable fabrication methods remain a challenge. Until recently, wax printing was the method of choice for many researchers, but with the growing scarcity of solid-ink printers, alternative technologies that are rapid, cost-effective, and scalable are needed. This presentation focuses on one such method: the air-gap PAD. Air-gap μPADs consist of hydrophilic paper zones attached to a hydrophobic backing with double-sided adhesive. These devices can be assembled by hand for prototyping purposes, but the primary appeal of our air-gap fabrication method is that it is compatible with the roll-to-roll methods used in large-scale manufacturing. This poster highlights the development of a roll-to-roll compatible fabrication method for air-gap μPADs and discusses its application to two μPAD designs: a paper-based titration device and a 12-lane μPAD for pharmaceutical screening in resource-limited settings. These two μPAD types were fabricated using both wax printing and the air-gap method and the resulting devices were tested to compare wetting behavior and colorimetric response. When the roll-to-roll air-gap technique is extended to additional device designs, it will provide a rapid and cost-effective fabrication method for large-scale manufacture of paper microfluidics.

Novel method of screening aqueous samples for PFAS

Yukun Jin (1), Aiden Flynn (1), Meghanne Tighe (1), Heather Whitehead (1), Graham F. Peaslee (1)
(1) University of Notre Dame

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made chemical compounds that were produced commercially since the 1940s and have been ubiquitously used in consumer products and industrial manufacturing processes. Examples include non-stick cookware, food packaging, textiles, cosmetics, batteries, and aqueous fire-fighting foams, all useful due to the hydrophobic and hydrophilic properties of PFAS. Through these widespread applications most PFAS ultimately end up in landfills in the US. Because PFAS have extreme persistence, they take years to degrade in the environment, and since most are soluble in water, they leach into ground water, pollute drinking water supplies, and are associated with adverse human health effects in many contaminated areas. We have developed a rapid screening method for all PFAS using a graphitized activated carbon (GAC) fabric to capture PFAS from water samples. When combined with a measurement of total fluorine by particle-induced gamma-ray emission (PIGE) spectroscopy, this can be a rapid and accurate screening tool to quantitatively measure total fluorine in aqueous samples. Since inorganic fluorides are added into some drinking water systems, the total fluorine signal is the sum of inorganic fluorides and organofluorines (such as PFAS) from any real-world water sample. We have developed a methanol rinse method to help remove PFAS from the GAC fabric but leave inorganic fluorides adsorbed on the GAC fabric after the extraction of water samples. In this way, we can determine not only the total fluorine concentration in a water sample, but also the fraction of organofluorine amount from PFAS. Results show that this is a rapid and affordable method to screen people's residential water samples for the presence of all PFAS at environmentally relevant levels of detection. This screening method could be used to evaluate the relevant efficiency for PFAS treatment processes.

Poster Presentations: Group A

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

Yuan Gao (1), Sophie R. Harvey (1); Vicki H. Wysocki (1)
(1) The Ohio State University

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 the 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. Our future plans include the characterization of ECD with more protein complexes with a wide range of oligomeric states and molecular weights. Multi-stage dissociation combined with ECD will also be used to study the structural and conformational change for the subunits of protein complexes.

Analysis of Protein Ligand Binding via Native Mass Spectrometry

William Moeller (1), Matthew Benedek (1), Mark Foster (1), Vicki Wysocki (1)
(1) The Ohio State University

Proteins carry out a vast array of functions that are critical for all forms of life. Proteins carry out these functions by interacting with a range of other molecules, including small organic compounds, transition metals, nucleic acids, or other proteins. Here, we investigate the interactions of several proteins with their binding partners using native mass spectrometry, an analytical technique which allows for the preservation of non-covalent interactions in the gas phase. (1) Cre recombinase. Cre, a protein enzyme with two domains, binds as a dimer to a specific DNA sequence and then synapses with a similar protein-DNA dimer to form an active tetrameric complex. The mechanism of the sequence specific DNA recognition and the coordination of DNA cleavage by the tetrameric complex are not fully understood. Understanding this interaction can potentially lead to a better understanding of how to direct DNA recombination to novel sequences. (2) S. pombe Loz1. The Loz1 protein functions by sensing intracellular zinc and at high zinc repressing the transcription of genes involved in zinc import. We use native mass spectrometry to quantify the thermodynamics of zinc sensing by Loz1, and its coupling to DNA binding. (3) B. subtilis Anti-TRAP (AT). AT is a small zinc binding protein that promotes production of the amino acid tryptophan by releasing the inhibitory effect of the TRAP protein. We use mass spectrometry to investigate the pH-dependent equilibrium of AT between functional trimeric and inactive dodecameric oligomeric states of AT.

Poster Presentations: Group A

Integration of nanoscale mixing tees with resistive-pulse sensing devices to improve detection limits of samples in low concentrations of supporting electrolyte

Quintin J. Brown (1), Michael P. Kappler (1), Adam Zlotnick (1), Stephen C. Jacobson (1)
(1) Indiana University

Resistive-pulse sensing is a label-free, nondestructive, single particle analysis technique that provides information about the size and surface charge of nanoscale particles. Resistive-pulse sensing requires a supporting electrolyte to act as a charge carrier that is displaced as a particle traverses a nanoscale pore, resulting in a change in resistance and corresponding current pulse. The pulse amplitude is proportional to the concentration of the salt, and as the electrolyte concentration is increased, the signal-to-noise ratio improves. Consequently, the concentration of the supporting electrolyte often limits the types of samples that can be analyzed by resistive-pulse sensing. Specifically, low salt concentration samples are not easily analyzed with traditional devices because of the low signal-to-noise ratio. We have designed a device with a nanoscale mixing tee integrated adjacent to the nanopore sensing region. One channel contains the sample, the other channel has a more concentrated solution of NaCl (>1 M), and the two solutions are mixed just prior to the detection region. The increase in salt concentration leads to a substantial increase in the signal-to-noise ratio. This new design effectively allows samples dissolved in low salt concentrations to be easily investigated with resistive-pulse sensing.

Observation of Reactive Oxygen Intermediates During the Oxygen Reduction Reaction on M-N-C Catalysts

Seth Putnam (1), Joaquin Rodriguez-Lopez (1)
(1) University of Illinois at Urbana-Champaign

Proton-exchange membrane fuel cells (PEMFCs) are an important renewable technology that convert oxygen and hydrogen to electricity. PEMFCs are easily scalable and could be used for both mobile and grid level electricity applications. Platinum-based catalysts are typically used at both the anode and the cathode to perform the Hydrogen Evolution Reaction (HER) and the Oxygen Reduction Reaction (ORR) respectively. However, the quantities of platinum necessary to catalyze the sluggish ORR has made PEMFCs economically nonviable. Fe-N-C catalysts are precious metal free catalysts that have been developed to replace platinum at the cathode by catalyzing ORR. Fe-N-Cs have been reported with catalytic activities that approach those of traditional Pt-based catalysts. Despite impressive activities, precious metal free catalysts tend to degrade faster than Pt-based catalysts. The exact mechanism by which Fe-N-C materials catalyze ORR and how the intermediates may contribute to their lack of stability is an area of debate. It is our goal to use multimodal Raman spectroscopy and scanning electrochemical microscopy (SECM) to interrogate the local production of various reactive oxygen species (ROS) intermediates during ORR. Using SECM, we have been able to observe the production of hydroxyl radicals and hydrogen peroxide in situ and to characterize the loss of Fe-N4 active sites after cycling by Raman spectroscopy. We intend to use surface interrogation SECM to quantify the production of these ROS and to use simultaneous Raman spectroscopy to observe adsorbed intermediates.

Poster Presentations: Group A

High Precision Compliant Mechanism for Scanning Electrochemical Microscopy

Nikita Lukhanin (1), Michael Pence (1), Oliver Rodríguez (1), Joaquín Rodríguez- López (1)
(1) University of Illinois at Urbana-Champaign

Scanning electrochemical microscopy (SECM) is a scanning probe technique that images the electrochemical reactivity of a surface. SECM can provide insight into materials for energy conversion and storage, notably in the study of organic redox-flow battery materials.1 Organic redox-flow battery materials have morphologies that require nanometer spatial resolution, which is often achieved in SECM with piezoelectric actuators; however, these actuators are costly and pose a major barrier to entry for potential SECM users. A compliant mechanism-based actuator offers an alternative to piezoelectric actuators at a fraction of the cost. A compliant mechanism is composed of a singular body with flexible joints, that can perform movements that would traditionally require multiple parts. Actuators driven by compliant mechanisms can be fabricated using readily available and cheap supplies, and they are capable of reliably achieving nanometer scale movements. We have employed a compliant mechanism-based actuator that can achieve nanometer-scale spatial movements for a cost that is 100 times less than the price of a common piezoelectric actuator found within SECMs. With the aid of finite element analysis, a set of compliant mechanisms have been developed that offer a single degree of freedom while reducing the motion of the end effector to have a resolution within the nanometer scale regime, with a step size of 0.7 nm. Applying a linear control algorithm (PID) in combination with low noise electronics has allowed for S-curve motion across a 5 mm range in a single stage. This method of motion counteracts environmental noise such as thermal expansion and low frequency vibrations while also tolerating manufacturing inconsistencies of the compliant mechanism itself. Experiments performed with ultra-micro electrodes (diameters of 25 μm or below) have shown approach of the electrode to within 0.2 radii from the surface reliably. The ultra-microelectrodes maintained a specified distance from surface while counteracting thermal expansion. We demonstrate how this new mechanism can be fit to the corresponding theoretical curve in real time, to better understand sources of error while running an experiment.

The ElectroLab: an integrated platform for high throughput characterization of redox-active materials

Oliver Rodriguez Martinez (1), Michael Pence (1), Nikita Lukhanin (1), Inkyu Oh (1), Hung Nguyen (1), Charles Schroeder (1), Joaquin Rodriguez-Lopez (1)
(1) University of Illinois at Urbana-Champaign

Electrochemical characterization of redox-active materials is knowledge and time intensive, making it incompatible with hight throughput testing. Here, we present a platform for automated electrochemical characterization focused on the use of ultramicroelectrodes and small solution volumes that allows us to obtain thermodynamic and kinetic parameters with minimal human interaction. The platform consists on three sub-systems: the software (a Python library, desktop and web applications), the electrochemistry (microfabricated electrochemical cells and electronics) and the fluid manipulation (3D printer-like robotic arm and digital microfluidics). Each sub-system can be used as a standalone product, allowing for maximum flexibility to interface with high throughput chemical synthesis systems.

Poster Presentations: Group A

Probing of Redox-active and Redox-inactive Neurotransmitters Using Dual Functional Nanoelectrodes

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Interface between two immiscible electrolyte solutions (ITIES) which provides a unique analytical platform for the detection of ionic species of biological interest such as neurotransmitters have been developed at nanoscale by Shen lab to detect redox inactive neurotransmitters. Carbon fiber electrodes are commonly used by various groups to detect redox active neurotransmitters such DA, serotonin and octopamine. We are developing a multifunctional nanoscale electrochemical platform to detect two groups of neurotransmitters i.e., redox-active and redox-inactive. Here we used dopamine (DA) as one example of redox active neurotransmitter and acetylcholine (ACh) as an example of redox inactive neurotransmitter. Detection of DA and ACh is made possible by a dual functional nanoelectrode composed of carbon and ITIES channels. DA was detected at nanometer-sized carbon electrode channel based on its oxidation. Acetylcholine being a redox inactive molecule was detected on the second channel of the dual channel nanopipette made of a nanoscale ITIES electrode. Micro/nano ITIES interface are generally formed at the tip of a borosilicate/quartz pipette, inner surface of which is rendered hydrophobic to be filled with an organic solvent by a method called silanization. In the process of developing dual functional nano-carbon-ITIES electrode as an alternative strategy, we explored the liquid silanization method for nanoscale ITIES and demonstrated that a stable cyclic voltammogram for tetrabutylammonium ion transfer across water/dichloroethane interface can be accomplished. The liquid silanization methods we developed lay the foundation for future development of dual channel multi-functional probe where one channel is nanoITIES.

Label-free high-content screening based on coherent anti-Stokes Raman scattering microscopy

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Methods capable of high-content screening (HCS) have been historically dominated by fluorescence. However, the chemical labels used in these methods rely on introduce significant challenges to analyzing live cells, such as perturbation to metabolic pathways, poor chemical selectivity, and limited detection channels. Nonlinear optical sources of excitation, notably coherent anti-Stokes Raman scattering spectroscopy (CARS), is a rapidly developing field with a label-free nature capable of successfully addressing the shortcomings of fluorescence spectroscopy in many experimental environments, while also presenting unique impediments of its own. Among these are the short working distance incompatible with microplates and transmission signal detection for achieving good sensitivity, restricting the application of CARS for HCS. We developed a label-free CARS HCS platform using a pulse-picking technology to acquire images in the epi direction with good sensitivity for live cells. This configuration allows the use of microplates and a surrounding incubation chamber for precision sample control and the simultaneous label-free chemical analysis of up to 64 conditions. This novel CARS-HCS platform allows automatic quantification and time-lapse monitoring of numerous conditions within a precision-controlled microenvironment.

Poster Presentations: Group A

Nano-DESI Mass Spectrometry Imaging of N-Linked Glycans in Tissue Sections

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Introduction: Glycans are important biomarkers of several diseases including cancer and diabetes. Most applications of nanospray desorption electrospray ionization mass spectrometry imaging (nano-DESI MSI) have focused on profiling metabolites, lipids, and proteins in tissues. Herein, we explore the potential of this technique for imaging of N-linked glycans. Nano-DESI MSI enables ambient liquid-extraction imaging of biological tissues. Imaging of N-glycans by nano-DESI MSI is of interest for glycomics analysis since it provides both relative abundance and spatial localization of these important biomolecules. We examine the localization of ~47 N-glycans in hepatocellular carcinoma tissue sections. Demonstrating nano-DESI MSI provides complementary information to other imaging modalities for N-glycans. Methods: Herein, we developed a method to profile N-glycans using nano-DESI MSI on FFPE prostate cancer tissue sections. Peptide N-glycosidase F (PNGaseF) is applied directly to tissue sections followed by incubation to release N-linked glycan species. Imaging experiments were performed on a Q-Exactive HF-X Orbitrap mass spectrometer using a custom designed nano-DESI source. Nano-DESI experiments were performed using a solvent consisting of 7:3 MeOH:H2O for optimal extraction of N-linked glycan species. Preliminary Data: Hepatocellular carcinoma tissue sections were ideal for method development because of the robust glycan signal and previous extensive profiling of the glycome. Using nano-DESI MSI, we observed ~47 N-linked glycans in prostate cancer tissue in positive ion mode. These results are comparable to matrix-assisted laser desorption (MALDI) MSI experiments, in which ~50 N-linked glycans have been previously observed. In nano-DESI MSI, N-linked glycans were observed as sodiated species, [M+Na]+. Meanwhile, glycans containing sialic acid residues are ionized as [M+2Na-H]+ species. Aside from the singly charged species, we detect doubly charged species in the form of [M+2Na]2+ and [M+3Na-H]2+ ions. The ability to generate multiply charged ions of glycans is advantageous for N-glycan sequence elucidation by MS/MS in untargeted analysis. Novel Aspect: Development of a new method for imaging of N-linked glycans in tissue sections using nano-DESI MSI.

Diffusion Mapping with Diffractive Optical Elements for Periodically Patterned Photobleaching

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Fourier transform fluorescence recovery after photobleaching (FT-FRAP) using diffractive optical elements (DOEs) is shown to support spatially resolved diffusion analysis in biologically relevant media. Characterization of diffusion is routine for the assessment of mobility in cell biology and lends insight into in vivo bioavailability of various drug products and biotherapeutics. Conventional point-bleach fluorescence recovery after photobleaching (FRAP) can measure diffusion over a micrometer scale and is noninvasive with low sample volume requirements, however conventional FRAP is complicated by sample heterogeneity, deviations from Gaussian bleach profile, and limitations in bleach-depth. FRAP performed with periodically patterned illumination followed by analysis in the spatial frequency domain has been shown previously to overcome many of these complications.1 The architecture for FT-FRAP was extended to 2D analysis of the diffusion tensor through simple modification of a commercial high-throughput FRAP system by integration of diffractive optical elements (DOEs) for patterned photobleaching, coupled with focal plane array detection.

Poster Presentations: Group A

Gas-phase Fragmentation of Viologen-based Host-guest Complexes

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Viologens are a class of molecules composed of a bipyridium center and two hydrocarbon-based side chains. The bypiridium center, which holds up to two positive charges has been widely used to create electrochromic devices. The mechanism of this reaction has been extensively studied in the condensed phase. However, it is difficult to explore this reaction in the gas phase because viologens are fairly unstable as doubly charged species. Previous studies have shown that gaseous doubly charged viologen species can be stabilized by incorporating them into an appropriate host molecule such as cyclodextrin or cucurbituril. A systematic study of the fragmentation pathways of these host-guest complexes provides first insights into the role of intermolecular interactions on their gas phase stability. Benzyl, heptyl and methyl viologen were the guest molecules used in this study, and typically a 15 μM solution of each species was prepared in water for MS analysis. 80 μM solutions of the host molecules (cucurbituril 6-8, **α**-, **β**- and **γ**-cyclodextrins) were prepared in a 1mM NaCl solution to improve the solubility of these compounds. MSn experiments were conducted in positive mode on a Thermo LTQ XL. We observed that the stability of doubly charged viologens in the gas phase is dependent on the identity of their side chains. For example, viologens with longer alkyl chains fragment a side chain as an alkyl cation, leaving a remaining singly charged viologen. In addition, by incorporating viologen into cucurbituril or cyclodextrins hosts we were able to stabilize the doubly charged viologen guest inside the nonpolar cavity of the host. By analyzing their fragmentation pathways, we determined that the stability of these supramolecular complexes towards fragmentation is dependent on the host-guest intermolecular interactions. Particularly, cucurbiturils bind more strongly to the guest than cyclodextrins and, upon fragmentation, we observe the formation of a singly charged cucurbituril/viologen complex. In contrast, fragmentation of cyclodextrin/viologen complexes results in separation of the host and guest molecules. In addition, the strength of the host-guest interaction is greatly influenced by the ability of the host to efficiently incorporate the guest. Overall, these results provide a step towards understanding the gas-phase stability of viologen based host-guest complexes.

Gas-phase Reactivity Study on Singlet Aryloxenium Cations

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Aryloxenium cations contain a formally positively charged, monovalent oxygen atom with an incomplete electron shell. They are key intermediates in electrochemical and chemical oxidation of phenols, and they are thought to persist in interstellar space. However, their chemical properties are difficult to study in solution due to challenges in generating them and their short lifetimes in solution. The aryloxenium cations were generated from synthesized precursors having a methyl or nitro group in the benzene ring of quinoline. These compounds were dissolved in carbon disulfide and were ionized by APCI to generate stable radical cations. The radical cations were transferred into the ion trap of the linear quadrupole ion trap mass spectrometer and subjected to collision activated dissociation to cleave off the methyl group or a nitrogen monoxide to generate the oxenium cations. We have studied the reactivity of different types of aryloxenium in the gas phase by using linear quadrupole ion trap mass spectrometers. Herein, we report the gas-phase reactivities of aryloxenium cations. Reactions of aryloxenium cations with dimethyl disulfide, allyl iodide and cyclohexane produced stable adduct formations, abundant allyl abstraction, hydride and proton abstraction respectively.

Poster Presentations: Group A

Mass spectrometry imaging of lipidome in a mouse model of Alzheimer's disease

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Lipids and fatty acids (FAs) are key components of the brain tissue involved in numerous cellular processes. Bulk lipidomics experiments have demonstrated alterations in concentrations of several FAs and lipids in Alzheimer's disease (AD). However, changes in the spatial distributions of AD biomarkers have not been comprehensively studied. Nanospray desorption electrospray ionization (nano-DESI) is an ambient ionization technique, which enables imaging of lipids and FAs with high sensitivity and spatial resolution. Herein, we use nano-DESI MSI to obtain spatial maps of lipids and FAs in brain tissues of 5xFAD mouse model of Alzheimer's disease. Our experiments reveal changes in the spatial distributions of several lipids and metabolites in AD brain compared to age-matched controls. We characterized the spatial distributions of >20 FAs and tens of phospholipids in both WT and 5xFAD sections. A majority of lipids and FAs show the same spatial localization and comparable abundance in both WT and 5xFAD brain tissues. Meanwhile, several species showed differences in abundances or/and spatial distributions between the two groups. For example, docosahexaenoic acid (DHA) showed the same spatial distribution in both the WT and 5xFAD tissues. However, its abundance decreased almost 2-fold in 5xFAD sections as compared to WT sections in isocortex, hypothalamus and hippocampal formation regions but remained unchanged in midbrain, thalamus, cortical plate and cortical subplate. This observation is consistent with the neuroprotective effect of DHA reported previously. Arachidonic acid (AA) showed similar spatial distributions as DHA. The amount of AA in the hippocampal formation region in the 5xFAD tissue is almost 2-fold lower than that in the WT tissue. Linoleic acid (LA) and oleic acid (OA) show a 2-fold and 1.5fold decrease, respectively, in the 5xFAD tissue. All the above FAs are known to prevent amyloid fibril formation. The observed decrease in the concentrations of these species in the AD brain is consistent with previously published bulk lipidomics data. FA (22:4), FA (22:1), and FA (22:2) all showed distinct distributions and decreased expression in the 5xFAD tissue in comparison with the WT tissue. In addition, several lysophospholipids showed alterations in the 5xFAD tissue. For example, LPA (20:2) decreased more than 2-fold in the 5xFAD tissue as compared to the WT tissue. LPS (14:0), LPE (18:1) and LPE (20:1), which showed a similar localization to the hypothalamus and thalamus regions, have a higher abundance in the AD brain as compared to the WT brain. Our results mapped variations of multiple metabolites and lipids in healthy and Alzheimer's disease tissues.

Poster Presentations: Group A

Intraoperative Assessment of IDH Mutation via Desorption Electrospray Ionization Mass Spectrometry to Aid Glioma Resection

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Glioma is a common and aggressive brain cancer, whose first-line treatment is tumor resection during surgeries. Currently, imaging modalities in clinics and intraoperative pathology cannot clearly delineate the tumor boundary for its gross total resection, which encourages the exploration of molecular diagnostics to provide near-immediate and accurate tumor diagnosis. Isocitrate dehydrogenase (IDH) mutation is a significant molecular biomarker of glioma with prognostic, diagnostic and therapeutic implications. Knowledge of IDH mutation during surgeries helps guide the extent of tumor resection, since more aggressive resection of IDH mutant tumors is associated with increased survival. Additionally, IDH mutation is unique to glioma tumors, suggesting its use as a potential surrogate to detect tumor infiltration. IDH mutation alters enzymatic pathways and produces an exclusive oncometabolite, 2-hydroxylglutaric acid (2HG). In current study, desorption electrospray ionization mass spectrometry (DESI-MS) is used to rapidly characterize the relative intensity of 2HG vs. its endogenous reference compound, glutamic acid, in brain biopsies to assess IDH mutation status during glioma surgeries. Since no sample preparation is required by DESI-MS, the analysis time per sample is as short as 1.5 min. Diagnostic performance of our intraoperative IDH mutation assay by DESI-MS has been evaluated using 116 core brain biopsies collected from 24 patients, which yields a sensitivity, specificity, and accuracy of 97%, 98%, and 98% respectively. When examining IDH status of biopsies near tumor margins, we have noticed that 88% margin biopsies (30 out of 34 biopsies) of IDH mutant tumors display great amounts of 2HG. This indicates significant residual tumor beyond MRI-defined surgical margins and suggests the promise of implementing our IDH mutation assay to define a clean margin for complete tumor resection.

Chemical Characterization of Brown Carbon in Atmosphere and Snowpack from the Colorado Rockies.

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Snow fall is a crucial source of fresh water, nutrients, and carbon for land ecosystems, especially in mountain regions. Deposits of atmospheric aerosols within snowpack are photochemically active, which influence the local land-atmosphere interactions. In terms of climate effects, snowpack is one of the highest light reflecting surfaces on Earth, and a key factor in Earth's radiative balance. The deposition of light-absorbing particles composed of brown carbon (BrC), black carbon (BC), and mineral dust significantly impacts the absorption of solar radiation. This results in decreased snow albedo and accelerates the rate of snow melt, which in turn influence the regional and global climate. Our study uses microscopy, optical measurements, and molecular characterization to investigate the optical properties and composition of BC and BrC deposited on the snowpack. The study employs real time aerosol sampling and monitoring at a Colorado field site using an aethalometer coupled with a CO2 probe. Particle and snow samples are collected for bulk composition analysis utilizing chemical imaging and molecular characterization techniques. We have found that the majority of light absorption comes from BrC with only minor absorption from BC and mineral dust.

Poster Presentations: Group A

Data-independent acquisition phosphoproteomics of urinary extracellular vesicles enables renal cell carcinoma grade differentiation

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Translating the research capability and knowledge in cancer signaling into clinical settings has been slow and ineffective. Recently, extracellular vesicles (EVs) have emerged as a promising source for developing disease phosphoprotein markers to monitor disease status. This study focuses on the development of a robust data-independent acquisition (DIA) using mass spectrometry to profile urinary EV phosphoproteomics for renal cell cancer (RCC) grades differentiation. We examined gas-phase fractionated (GPF) library, direct DIA, forbidden zones, and several different windowing schemes. After the development of a DIA mass spectrometry method for EV phosphoproteomics, we applied the strategy to identify and quantify urinary EV phosphoproteomes from 57 individuals representing low-grade clear cell RCC, high-grade clear cell RCC, chronic kidney disease (CKD), and healthy control (HC) individuals. Urinary EVs were efficiently isolated by functional magnetic beads and EV phosphopeptides were subsequently enriched by PolyMAC. We quantified 4,057 unique phosphosites and observed that multiple prominent cancer-related pathways, such as ErbB signaling, renal cell carcinoma, and regulation of actin cytoskeleton, were only upregulated in high-grade clear cell RCC, while those correlated with a higher survival rate were elevated in low-grade clear cell RCC only. These results show that EV phosphoproteome analysis utilizing our optimized procedure of EV isolation, phosphopeptide enrichment, and DIA method provides a powerful tool for future clinical applications.

High-throughput label-free opioid receptor binding assays using automated desorption electrospray ionization mass spectrometry (DESI-MS)

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The identification of novel small-molecule candidates with high analgesic properties but reduced side effects (e.g. addiction) is of high importance in the context of the current opioid epidemic. To facilitate the discovery of such new non-addictive analgesic candidates, and overcome the multiple drawbacks associated with current radioligand binding assays (e.g. cost, safety concerns, versatility), we focused on the development of a label-free approach for probing the relative binding affinity of small molecules towards opioid receptors. Here we demonstrate a competitive ligand binding assay towards μ and **δ** opioid receptors developed using an automated high-throughput platform based on desorption electrospray ionization mass spectrometry (DESI-MS). In these assays, leucine enkephalin (LeuEnk, utilized as competitive ligand), testing compounds, and commercial membrane preparations of opioid receptors are incubated together (1 h, 37 °C) in Tris buffer pH 7.4. After incubation, DADLE (a methylated analog of LeuEnk used as internal standard for quantification) is added, and the mixture is rapidly filtered. The amount of free LeuEnk is finally quantified by high-throughput (<1 second per sample) DESI-MS using minimal sample volumes (50 nL) and it is used as an indicator of the binding strength of the testing compounds. Several dose response relationships for multiple known opioids (e.g. naloxone, naltrindole, buprenorphine, PZM-21) demonstrate the agreement between our novel label-free methodology and the traditional approaches based on scintillation counting.

Poster Presentations: Group A

Imaging and controlling chemicals in biological samples using real-time precision opto-control (RPOC)

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The precise control of biochemical reactions in live cells is a long-sought goal in biological sciences. Conventional approaches such as chemical treatment and genetic methods lack spatial and temporal precision. Current approaches using lasers manipulation such as optical tweezers, ablation, and optogenetics require prior knowledge of the sample and manual selection of a few manipulation targets. For live biological samples, dynamic processes of highly mobile organelles make the slow manual selection of targets difficult for the meaningful capture and control of complex biochemical processes. To combat this challenge, we develop a real-time precision opto-control (RPOC) technology that allows the user to detect and manipulate dynamic cellular processes simultaneously and automatically in real time. It operates by comparing the generated optical signals during laser scanning with preset voltage values using comparator circuitry to control another laser beam that triggers chemical reactions. The control laser is coupled from the 1st order output of an acousto-optic modulator (AOM) with a nanosecond response time. This control laser is only activated at the pixels of interest. This creates a feedback loop that is faster than the pixel dwell time for real-time opto-control. RPOC allows us to control chemical processes with high spatial accuracy, in real-time, and with high chemical selectivity. We can achieve higher-order target selection processes such as bandpass filters and digital logic for better chemical selectivity by combining multiple comparator circuits. We demonstrate precision control of the states of photochromic molecules at different parts of the cell. We also employ RPOC with photoswitchable microtubule polymerization inhibitors to control intercellular dynamics of microtubule and lipid droplets.

Poster Presentations: Group A

Multiple Cation Switching in Peptides and Proteins: Replacement of Labile Protons with Metal Ions via Gas-Phase Ion-Ion Chemistry

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ESI of peptides/proteins generally yields ions with a net excess of protons. At relatively high salt concentrations, cations with a few metal ions are also observed. However, it is very rare to observe ions in high abundance in which all excess positive charge is accounted for by metal ions. Here we demonstrate that it is possible to replace all excess protons (and more) with metal ions via gas-phase ion/ion chemistry. The total number of protons that can be replaced with metal ions is limited to the number of 'labile' hydrogens. In addition to counting labile hydrogens, this work allows for the study of the fragmentation behavior of polypeptide ions with a wide range of proton/metal ion combinations. This study uses a TripleTOF 5600 quadrupole time-of-flight mass spectrometer (SCIEX, Concord, ON, Canada) that has been modified for ion-ion reactions. Anions and cations were alternately pulsed using dual nano-electrospray ionization for sequential injection. Sodium acetate (NaOAc) and other metal-bound acetate compounds were used as the anion reagent, and peptides (ex: GGGGGGGR, GGGMGGGR) were used as the cation analytes. Different salt cluster sizes are selected in Q1 and stored in q2. The peptide is then mass selected in Q1, then mutually stored in q2 with the salt cluster. The resulting product ions are then isolated and fragmented in q2 with ion trap collision-induced dissociation (IT-CID). Current research focuses on simple peptides and NaOAc clusters. The ion-ion reaction products unique to salt clusters are long-lived complexes of the peptide and salt cluster with neutral losses (NL) of acetic acid (HOAc, NL = 60 Da). After a certain number of proton-metal exchanges, the loss of HOAc competes with the loss of NaOAc (NL = 82 Da) until only NaOAc losses are seen. Once all acetates have left, the resulting product is a peptide saturated with Na. In order to determine where these metals are exchanging with protons on the peptide, this study has explored a number of variables that affect the number of proton-metal exchanges, such as number of residues, side chain compositions, methyl-esterification of the C-terminus. DFT modeling is also used to predict whether an exchange will occur at a peptide site using proton and metal affinities of the ligand and the site.

Poster Presentations: Group A

Simultaneous and Spontaneous Oxidation and Reduction in Microdroplets by the Water Radical Cation/Anion Pair

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Chemical reactions in micron-sized droplets under ambient conditions are often orders of magnitude faster than the equivalent bulk reactions. The observed reaction acceleration is explained by the peculiar environment at the droplet air/solution interface, where reactants are only partially solvated and which is characterized by a high electric field. Both these features strongly influence the rates of reactions intrinsic to the chosen reagents, but they may also activate solvent molecules (e.g. water) at the interface and this could result in microdroplet-specific reactions, especially their intrinsic redox properties. Spontaneous oxidation or reduction without external oxidants or reductants has been reported. One explanation for the active species is the dissociation of the radical cation/anion pair ($H_2O^{+\cdot}$/ $H_2O^{-\cdot}$), recently argued to exist in pure bulk water, to provide the free radical cation and radical anion. However, simultaneous oxidation and reduction in microdroplets has not yet been reported; such an observation would support the radical pair as the source of reactive species in water microdroplets. Here, we report the concurrent conversions of several phosphonates to phosphonic acids by reduction (R-P -> H-P) and to pentavalent phosphoric acids by oxidation. The experimental results suggest that the active reagent is the water radical cation/anion pair. The water radical cation is observed directly as the ionized water dimer while the water radical anion is only seen indirectly though the spontaneous reduction of carbon dioxide to formate. This result supports a double-layer model where the strong electric field aids the dissociation of the water radical cation/anion pair to free ions as well as enrichments of oppositely charged ions in separate layers. As a result of oxidizing and reducing layers being established, oxidation and reduction occur simultaneously. The spontaneous conversion of phosphonate, involving oxidation and reduction as well as hydrolysis, could provide new insights into its biological transformations.

Poster Presentations: Group A

Miniature Mass Spectrometer-Based Point-Of-Care Methods for Analysis of Drugs and Biomarkers in Biofluids

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Current emphasis in drug and biomarker detection is shifting towards point-of-care (POC) testing to empower clinicians and simplify healthcare delivery. While immunoassays and colorimetric tests have successfully been implemented for making on-site measurements, trace analysis is best done by mass spectrometry because of its wider molecular applicability and sensitivity. Improvements in ambient ionization methods have helped realize the power of miniature mass spectrometers, thus extending their utility to clinically relevant POC measurements. Here, we expand the capabilities within this space by demonstrating rapid and accurate quantitative analysis of (i) cabotegravir and/or rilpivirine in blood for HIV in treatment (ii) phosphatidylethanol (PEth) in blood as a biomarker for alcohol abuse. To assess the reliability with which biomarkers and drugs in biofluids could be measured using a of portable mass spectrometer, spiked samples of various drugs and biomarkers targeting several different applications. Clinically relevant concentration ranges were chosen for these analyses using paper spray ionization and the Mini-12. The first application was directed at the measurement of levels of cabotegravir and/or rilpivirine, drugs used for HIV prevention and treatment. Quantitative performance was achieved over the entire clinically relevant range of the drugs in whole blood, with limits of quantitation (LoQs) of cabotegravir and rilpivirine measured as 750 ng/mL and 20 ng/mL, respectively. The second major application involved development of a method to quantify Phosphtidylethanol (PEth), a downstream metabolite which is formed only when alcohol is consumed. Concentrations of PEth in blood reflect alcohol consumption 2 - 4 weeks prior to collection and serve as a promising marker for quantifying a person's use of alcohol. From a calibration curve, good quantitative performance was obtained with the LoQ of 50 ng/mL. Concentrations corresponding to significant consumption (20 - 199 ng/mL) and heavy consumption (>200 ng/mL) could readily be distinguished. The speed (< 4-minute turnaround time), portability, sensitivity, low power consumption, and specificity offered by the Mini-12 instrument with the demonstrated examples means that it represents a simple and accurate platform for measuring drug and biomarker levels using small volumes of biofluid sample.

Poster Presentations: Group A

Understanding the Reactivity of Undercoordinated Ions on Surfaces Using Ion Soft Landing

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Soft-landing of mass-selected ions onto surfaces is ideally suited for the purification of molecules from complex mixtures and preparation of well-defined ionic interfaces. Fragment ions generated in the gas phase are reactive species that demonstrate unique and largely unexplored reactivity in condensed phase. This reactivity is distinctly different from both their reactivity as isolated gaseous ions and fully solvated species in condensed phase. Our study indicates that this reactivity is dominated by electron transfer, ligand exchange, and binding of SAM molecules. Understanding the reactivity of fragment ions on surfaces opens new directions for generating condensed phase products for applications in catalysis, photovoltaics, and materials design. In this study, fragment ions are generated in a custom-built high-flux ion soft landing instrument. Briefly, ions generated using electrospray ionization (ESI) are transferred into a collision cell. Fragment ions formed in the collision cell are mass-selected using a quadrupole mass filter and deposited onto a self-assembled monolayer (SAM) of either a methyl-terminated alkyl thiol on gold (HSAM) or fluorinated alkyl thiol (FSAM). The system enables co-deposition of ions of different polarity or different m/z. A custom-built script is used to control the number of ions deposited in each soft-landing segment. This capability is used to examine the extent of reaction when ions are codeposited in submonolayer or monolayer segments. The reaction products generated on a surface are analyzed using nano-DESI mass spectrometry on an Agilent 6556 IM-QTOF instrument. We focused our studies on the reactivity of the undercoordinated organometallic fragment ions soft-landed onto SAM surfaces. We used ruthenium (II) trisbipyridine Ru(bpy)32+ as a model system that undergoes ligand loss in the gas phase to generate the undercoordinated Ru(bpy)22+ fragment. When soft-landed onto HSAM, this fragment ion generates several reaction products observed using nano-DESI-MS analysis. Specifically, we observe ligand exchange between soft-landed Ru(bpy)22+ fragments generating the fully coordinated Ru(bpy)32+ species, charge reduction generating singly charged Ru(bpy)2+, and reaction with thiol molecules of the HSAM, which solvate the fragment ion on the surface generating [Ru(bpy)2(HSAM)-H]+. We also examined the reactivity of soft-landed fragment ions of undercoordinated Ni-bipyridine organometallic complexes. In these experiments, Ni(bpy)32+ generated using ESI were subjected to CID to generate fragments corresponding to the loss of one ligand. For these species, electron transfer and ligand exchange are the dominant reaction pathways. When the undercoordinated Ni(bpy)22+ ion is deposited onto a surface it remains mostly stable on the surface, a behavior that is striking different from the observed for Ru(bpy)22+. We hypothesize that the stability of Ni(bpy)22+ on the surface is due to the possibility of the tetracoordinated Ni-metal centers to form stable square planar geometries. Overall, we demonstrate that the unique reactivity of fragment organometallic ions after being deposited or codeposited onto SAM surfaces may be used to generate novel organometallic compounds not accessible by conventional gas or condensed phase syntheses.

Poster Presentations: Group A

Multiagent Consensus Equilibrium (MACE) in Molecular Spectral Analysis

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Soft-landing of mass-selected ions onto surfaces is ideally suited for the purification of molecules from complex mixtures and preparation of well-defined ionic interfaces. Fragment ions generated in the gas phase are reactive species that demonstrate unique and largely unexplored reactivity in condensed phase. This reactivity is distinctly different from both their reactivity as isolated gaseous ions and fully solvated species in condensed phase. Our study indicates that this reactivity is dominated by electron transfer, ligand exchange, and binding of SAM molecules. Understanding the reactivity of fragment ions on surfaces opens new directions for generating condensed phase products for applications in catalysis, photovoltaics, and materials design. In this study, fragment ions are generated in a custom-built high-flux ion soft landing instrument. Briefly, ions generated using electrospray ionization (ESI) are transferred into a collision cell. Fragment ions formed in the collision cell are mass-selected using a quadrupole mass filter and deposited onto a self-assembled monolayer (SAM) of either a methyl-terminated alkyl thiol on gold (HSAM) or fluorinated alkyl thiol (FSAM). The system enables co-deposition of ions of different polarity or different m/z. A custom-built script is used to control the number of ions deposited in each soft-landing segment. This capability is used to examine the extent of reaction when ions are codeposited in submonolayer or monolayer segments. The reaction products generated on a surface are analyzed using nano-DESI mass spectrometry on an Agilent 6556 IM-QTOF instrument. We focused our studies on the reactivity of the undercoordinated organometallic fragment ions soft-landed onto SAM surfaces. We used ruthenium (II) trisbipyridine Ru(bpy)32+ as a model system that undergoes ligand loss in the gas phase to generate the undercoordinated Ru(bpy)22+ fragment. When soft-landed onto HSAM, this fragment ion generates several reaction products observed using nano-DESI-MS analysis. Specifically, we observe ligand exchange between soft-landed Ru(bpy)22+ fragments generating the fully coordinated Ru(bpy)32+ species, charge reduction generating singly charged Ru(bpy)2+, and reaction with thiol molecules of the HSAM, which solvate the fragment ion on the surface generating [Ru(bpy)2(HSAM)-H]+. We also examined the reactivity of soft-landed fragment ions of undercoordinated Ni-bipyridine organometallic complexes. In these experiments, Ni(bpy)32+ generated using ESI were subjected to CID to generate fragments corresponding to the loss of one ligand. For these species, electron transfer and ligand exchange are the dominant reaction pathways. When the undercoordinated Ni(bpy)22+ ion is deposited onto a surface it remains mostly stable on the surface, a behavior that is striking different from the observed for Ru(bpy)22+. We hypothesize that the stability of Ni(bpy)22+ on the surface is due to the possibility of the tetracoordinated Ni-metal centers to form stable square planar geometries. Overall, we demonstrate that the unique reactivity of fragment organometallic ions after being deposited or codeposited onto SAM surfaces may be used to generate novel organometallic compounds not accessible by conventional gas or condensed phase syntheses.
Originally developed for computational image reconstruction1, MACE is shown in this work to support the combined integration of quantum chemical calculations with experimental observations in a single equilibrium evaluation. Vibrational and rotational spectroscopy provide optical “fingerprints” of chemical composition, with spectroscopic signatures routinely used in hyperspectral chemical microscopy. Interpreting molecular spectroscopy is often aided by quantum chemical calculations, in which molecular bonding information is used to predict spectral signatures. Comparisons between measured and calculated spectra is often done qualitatively and empirically, with few widely accepted metrics for success or failure in spectral agreement. In this word, we implemented MACE to integrate experimental observables as constraints in the simultaneous evaluation of molecular structure by multiple computational architectures. MACE is founded on simultaneously determining the equilibrium point between multiple experimental and/or computational agents; the returned state description (e.g., atomic coordinates for molecular structure) represents the intersection of each manifold and is not equivalent to the average optimum state for each agent. The moment of inertia, determined directly from microwave spectroscopy measurements, serves to illustrate the mechanism through which MACE evaluations merge experimental and quantum chemical modeling. MACE results reported combine gradient descent optimization of each ab initio agent with an agent that predicts the chemical structure based on root-mean-square deviation of the predicted inertia tensor with experimentally measured moments of inertia. Successful model fusion for several small molecules was achieved as well as the larger molecule solketal. Fusing a model of moment of inertia, an underdetermined predictor of structure, with low-cost computational methods yielded structure determination performance comparable to standard computational methods such as MP2/cc-pVTZ and greater agreement with experimental observables. Building on the successful extension of MACE beyond computational imaging into quantum chemistry, we further explored applications for vibrational spectral analysis in hyperspectral imaging. Connecting spectroscopy to chemical composition is often aided by dimension reduction, in which spectra are cast in lower-dimensional “feature-spaces” to simplify analysis. Every dimension reduction approach brings its own advantages and disadvantages. Rather than select one among many possible dimension reduction algorithms, MACE was implemented to identify the optimal feature-space balancing multiple simultaneous algorithms. By using MACE framework to combine GALDA and PCA, we were able to manually adjust the separation of each class of data (defined as resolution) and overfitting (defined as separation performance difference between training and testing), so that the overfitting problem in dimension reduction should be reduced to a small value, while the resolution is retailed at high value. Initial demonstration of GALDA MACE method for Raman Spectra dimension reduction is presented, along with a critical discussion of the figures of merit and future developments.

Poster Presentations: Group B

Low-cost screening test for lead in water based on activated carbon felt (ACF) capture and x-ray fluorescence (XRF) detection: introducing on-site drying method and investigating accuracy of test among different water types

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Recent news reports indicate that dangerous levels of lead have been found in thousands of homes and schools across the US. This is caused by corroding lead service lines and/or lead plumbing fixtures in the home. In many states, health authorities encourage residents to have their home tested for lead. To identify the lead risks in a home, a resident can request a Lead Inspection Risk Assessment (LIRA). A LIRA is conducted by a certified risk assessor who uses a handheld x-ray fluorescence spectrometer (XRF) to read the lead content in the paint samples of the home. On the other hand, water samples cannot be read on site, but must be transported back to the laboratory. On-site reading is not possible since the XRF lacks the sensitivity to directly read the lead content at the low, but nonetheless toxic levels at which it is present in the water. Moreover, since a laboratory test for lead in water costs between $15-$50, a LIRA accepts only one water sample for testing. A low-cost method for reading the lead in water using an XRF was previously developed .This method overcomes the low sensitivity of the XRF by capturing and pre-concentrating the lead from the water using an activated carbon felt (ACF) filter. The ACF filter is fitted into a cap of a 2 liter bottle, the bottle is filled with water and the water is allowed to drain out through the filter. The filter is then removed, dried in a convection oven, and the lead content is analyzed on the XRF. Here, we describe development and validation of an on-site drying method that enables in-home screening of tap water to be carried out during a LIRA. The filter(s) can be dried and analyzed with the XRF on-site and the result can be immediately communicated to the resident. Multiple water samples can be screened at a minimal cost of 20 ¢ per additional test, helping to identify which parts of the house have lead plumbing fixtures. We systematically varied water quality parameters such as pH, water hardness, chlorine content and presence of humic substances, to see how they affected the adsorption of lead onto the ACF. The outcomes of these experiments together with measures to improve the accuracy of the test are presented in this work. Having an accurate, semi-quantitative test for lead at 1% of the cost of the present lab test allows for a large number of water samples to be tested from different faucets and/or parts of the plumbing system in a home; the sampling could also be carried out over longer time spans or during periods when municipal water systems are undergoing repair work that can release lead from passivation layers in the pipes. This will enable residents to a) identify if there is a lead risk from their water b) determine which part of the plumbing poses the risk (fixtures or service line) and c) take remedial measures such as replacing the plumbing and/or using a drinking water filter. These measures prompted by the improved ACF-XRF water test would reduce the number of persons, especially children, being lead poisoned in the US.

Poster Presentations: Group B

Detection of particulate lead in drinking water through the use of activated carbon felts

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Recent water crises in cities such as Flint and Newark have shown that lead in drinking water is still a concern in many US locations. Previously, the University of Notre Dame Lead Innovation Team had developed a process for quantifying lead in tap water utilizing an activated carbon felt (ACF) filter and X-Ray Fluorescence spectroscopy (XRF). This ACF filter has been shown to effectively trap soluble lead, but its effectiveness at capturing particulate lead, which is also common in tap water, was unknown. This work has studied the ACF's ability to capture particulate lead orthophosphate in the size range of 100 nm and compared it to the performance with soluble lead. Further experiments also tested the capture efficiency at a variety of pHs. Results have shown that newly received ACF captured lead at a lower efficiency than the original ACF used to develop this testing process. In response, methods to reactivate the functional groups in the ACF were investigated in an effort to improve the capture efficiency of both soluble and particulate lead. Efficient capture of both lead types would prove the robustness of this low-cost, convenient test method in screening water sources for lead contamination.

Genetically Based Migration of Microorganisms in an Electrophoretic Field

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Microbial mixtures can be separated via Capillary Zone Electrophoresis (CZE) due to their characteristic electrophoretic mobility observed across different species. In CZE, separation is based on the size to charge ratio of the analyte and altering this ratio will lead to changes in electrophoretic migration of microbes. Specific proteins, lipids and carbohydrates expressed on or near the surface of a microorganism should heavily influence the specific migration patterns. The genetic factors contributing to this separation of microbes by CZE is unknown. Determining the genetic basis of separation will lead to identification of genes that can be used in development of novel CZE based genetic tools for high-resolution bacterial separations and electrophoretic screens. Flagella are abundant hetero-polymeric protein filaments that can extend a significant distance (~μm) from bacterial cells and are responsible for swimming motility observed in bacteria like Escherichia coli. Due to their abundance and localization, they are an attractive target to alter the size to charge ratio of the bacterial cells and their subsequent electrophoretic migration. To investigate the effect of flagella on CZE migration, E. coli MG1655: a flagellated motile bacterium was subjected to deflagellation via deletion of selected flagellar synthetic genes. Mutants of MG1655 with fliC and fliD gene deletions were constructed using a Lambda Red recombinase system and the deletions were confirmed via PCR and Sanger sequencing. The motility of the wild type (WT) and mutant strains was confirmed by a culture media-based motility assay. Additionally, physical deflagellation of MG1655 was performed by mechanical shearing of flagella via passing the cells through a syringe needle repeatedly. Deflagellated bacterial samples were then subjected to CZE combined with automated fraction collection and fractions were deposited on to a media plate where altered migration patterns were observed for both fliC and fliD mutants compared to the WT MG1655.

Poster Presentations: Group B

Enrichment of Cysteinyl-Peptides with Bismuth Complexes for Small Protein Discovery

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The 'dark proteome' refers to the subset of proteins that remain unknown to the scientific community. Small proteins (sProts) make up a significant portion of the 'dark proteome' as they are challenging to detect for several reasons. Small proteins are defined as containing <50 amino acids, are often missed during automated gene-annotation, and discarded in traditional proteomics sample preparation methods. Additionally, sProts produce few proteolytic fragments and lack typical biochemical properties, making detection through mass spectrometry a challenge. Phenotypic roles for these proteins are difficult to determine due these limitations. New methods must be adapted to enrich and detect small proteins. sProts are highly represented in mycobacterial species, in particular including the causative agent of human tuberculosis, M. tuberculosis, and the non-pathogenic, M. smegmatis. We previously discovered and partially characterized a group of regulatory cysteine-rich sProts in Mycobacteria, however the majority of them were not confirmed by mass spectrometry. This suggests that targeted enrichment of sProts that advantage of predicted properties will allow for improved small protein discovery. Due to its thiophilic nature, we hypothesize that bismuth (Bi3+) will quickly and selectively capture cysteine residues, enabling ligand-free enrichment for analysis. We have developed two methods to enrich cysteinyl-peptides using Bi3+: immobilized metal affinity chromatography (IMAC) and pnictogen-mediated-peptide pelleting. We were able to generate stable Bi-NTA resin for cysteinyl-peptide selection, verified with XRF. Additionally, we have used BiBr3 to precipitate out cysteinyl-peptides from solution due to the high density of Bi metal and general insolubility in aqueous solutions. We utilized the Cys-rich protein BSA (35 cysteines) as a positive control to demonstrate enrichment and are performing enrichment on clarified cell lysates of Mycobacterium.

Improving the idPAD: New Lane Test Specific for the Identification of Methamphetamine

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As drug overdose rates continue to rise in the United States, there has been a growing emphasis on drug checking. Street drugs often contain contaminants and other illicit substances that the user is not aware of, but new testing devices are able to reveal the true composition of a sample. The current illicit drug paper analytical device (idPAD) has the ability to run a library of 12 colorimetric tests at a time, identifying a selection of illicit drugs, distractor substances, and cutting agents commonly found in street drug samples. In order to improve the accuracy and detection abilities of the idPAD, a new lane test is being developed which specifically recognizes secondary amines, such as methamphetamine. This test utilizes the Simon reaction, in which the systematic addition of acetaldehyde, sodium nitroprusside, and sodium carbonate produces a bright blue color change in the presence of the secondary amine. However, the volatility of acetaldehyde highly reduces its stability on paper, necessitating its storage in another form. The acetaldehyde ammonia trimer is a trimeric structure formed in a reversible reaction between ammonia and acetaldehyde. Reacting this trimer with acetic acid at pH 5 results in optimal acetaldehyde formation. Therefore, on paper, a sequential reaction of acetic acid, acetaldehyde ammonia trimer, methamphetamine, aqueous sodium nitroprusside, and sodium carbonate produces the same bright blue color change. Tests of primary and tertiary amines produced negative results. The limit of detection for this reaction by mass is 1% methamphetamine/ 99% lactose. Further studies regarding the stability of the acetaldehyde ammonia trimer are ongoing.

Poster Presentations: Group B

Electrochemical Stability of N-heterocyclic Carbene Monolayers: an EC – SERS Approach

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(1) University of Notre Dame, (2) University of Tennessee, Knoxville, (3) Johns Hopkins University School of Medicine

N-heterocyclic carbene (NHC) monolayers are the focus of intense recent interest owing to their ability to functionalize metal surfaces forming high–performance catalysts, sensors, and electronics. In particular, the modularity of carbene ligands and broad surface compatibility render these ligands useful for advanced electrochemical techniques, such as the electroreduction of CO2 or functionalization of gold electrode biosensors. Despite the promising nature of NHC surfaces, the fundamental electrochemical limitations of NHC surfaces on gold are unknown. For this reason, an in situ technique capable of probing molecular changes in NHC surfaces while modulating the electrochemical potential would be highly desirable. To this end, an electrochemical surface-enhanced Raman spectroscopy (EC-SERS) technique was developed to monitor NHC monolayers on roughened gold electrode surfaces. Changes in the resulting surface chemistry were monitored using SERS after cyclic voltammetry (CV) sweeps to increasingly positive or negative voltage windows. SERS spectra illustrate the remarkable stability of carbene monolayers as the NHC signals remain largely unchanged in potential windows relevant for electrochemical biosensors.

Surface Enhanced Raman Spectroscopy Study of Gold to Carbon Bond of N-Heterocyclic Carbene Ligands on Gold Surfaces

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N-heterocyclic carbene (NHC) ligands are an exciting new ligand for metal surface passivation with great potential for biomedical and electronics applications. The gold to carbon bond vibrational mode for NHC ligands was previously assigned to a mode at around 450 cm-1. In this study, we focused on the low frequency modes between 400 to 900 cm-1 in SERS spectra of several benzimidazolium carbene scaffolds chemisorbed to 60 nm gold nanoparticles. The SERS spectra reveal that several spectral features are conserved despite changing the substituents in the 5’ position or the side-groups. These experimental results were further augmented with theoretical calculations which revealed that the vibration modes in this region are not pure gold to carbon modes. Unlike previous literature reports, these results illustrate that the gold to carbon vibrations are but a component of vibrational modes which are heavily dependent on the carbene structure.

Poster Presentations: Group B

Characterizing the Stability of AAV Capsids with Variable Temperature-Charge Detection-Mass Spectrometry (vT-CD-MS) and Surface Induced Dissociation (SID)

Marius Kostelic (1), Michael T. Marty (2), Vicki Wysocki (1)
(1) The Ohio State University (2), University of Arizona

Adeno-associated virus (AAV) capsids are currently used as gene therapy and vaccine delivery systems. However, a bottleneck in their quality control is measuring the stability of DNA filled AAVs. Typically, AAV stability is measured with differential scanning fluorimetry (DSF) which gives a melting temperature (Tm) for an AAV capsid, but DSF cannot differentiate between the stability of different AAV subpopulations in the same sample, which are commonly found in biopharmaceutical AAVs. To characterize the solution phase stability of different AAV subpopulations in the same sample and measure the dissociation of DNA cargo from the capsid, we used variable temperature electrospray ionization mass spectrometry (vT-ESI-MS)—which characterizes solution phase unfolding of intact protein complexes by controlling the temperature of the ESI needle—with charge detection-mass spectrometry (CD-MS), which can measure levels of empty and filled AAVs based on their difference in mass. Furthermore, we used surface induced dissociation (SID) to fragment Filled AAV capsids to isolate and measure the incorporated DNA. We found that vT-CD-MS provided Tm¬ ranges for empty AAVs and the temperature of DNA ejection (TE) for filled AAVs. Then, we found that SID, unlike collision induced dissociation, offers enough energy to fragment intact capsids with incorporated DNA. This provides a useful tool for characterizing the structure of AAV capsids and offers a new quality control method for measuring the DNA cargo, which will further AAV therapy development.

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

Zihao Qi (1), Charles Bell (1), Vicki Wysocki (1)
(1) The Ohio State University (

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 monitor the chemical kinetics for the formation of complexes. These results will provide better insights on DNA repair, which will lead to further therapeutic development to treat various cancers.

Poster Presentations: Group B

Legacy and Emerging Contaminants in North American Herring Gull (Larus argentatus) Serum from the Laurentian Great Lakes

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Herring gulls (Larus argentatus) are a sentinel species for the Laurentian Great Lakes ecosystem. They are apex predators that bioaccumulate legacy pollutants through aquatic and terrestrial food sources. Therefore, concentrations of pollutants in herring gulls can be used to elucidate general trends in the concentrations and movements of legacy chemicals throughout the Laurentian Great Lakes. Herring gull blood samples were collected between 2010 and 2021 in Michigan near Lake Huron, Lake Michigan, and Lake Erie. Target chemicals were extracted from the blood serum through liquid-liquid extraction followed by solid-phase chromatography. Flame retardants were analyzed using GC-MS EI (electron ionization) while pesticides and PCBs (Polychlorinated Biphenyls) were analyzed using GC with an ECD (electron capture) detector. Preliminary results suggest a widespread accumulation of BDE congeners BDE-47, 99, and 153 in herring gull serum. All bird serum contains PCBs, of which the concentrations are dominated by congeners 118 and 180. Furthermore the pesticides and pesticide byproducts, p,p'-DDE (Dichlorodiphenyldichloroethylene), HCB (Hexachlorobenzene), Octachlorostyrene, and Dieldrin are present in numerous samples. These data, in conjunction with existing data on legacy and emerging chemicals in herring gulls, contribute to our understanding of the spatiotemporal trends of legacy and emerging contaminants in the Laurentian Great Lakes Region.

Electrochemical Studies of species adsorbed at electrodes: probing instabilities and in situ changes

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Due to the anthropogenic release of carbon dioxide (CO2), our planet faces a dire situation of rising temperatures and mass global climate change (1,2,3,4). To meet the Paris Agreement's 1.5°C global warming target, it is imperative to not only lessen carbon emissions but also to sequester already emitted carbon dioxide (1,2,3,4). Thus, an emerging field of research is discovering methods to capture atmospheric carbon dioxide. The state-of-the-art methodologies for the direct-air capture methods of CO2 are redox-active organic molecules (ROAs), electrochemically mediated amin regeneration (EMAR), and inorganic chemisorbents (IC) (1,3). These methods can readily capture CO2, but there is a hefty energy requirement (ROAs: 40-200 kJ/mol, EMAR: 101-242 kJ/mol, IC: >800°C) to release the captured CO2 for subsequent captures (1,3,4). Our objective is to create an easily reversible system that is less energy-intensive (3-12 kJ/mol) via an electrostatic charge transfer mechanism at modified electrode surfaces. Current work is on discovering the optimal system and conditions to modify electrode surfaces for our purpose. Experiments have been conducted on three distinct modified electrode types: (1) SAMs on gold electrodes, (2) molecules with pi-pi stacking interaction or (3) electrochemically grafted molecules on glassy carbon and graphene electrodes. These surface modifications are studied using spectroelectrochemistry. The spectroscopic techniques utilized include surface-enhanced Raman scattering (SERS), surface plasmon resonance (SPR), and surface-enhanced infrared absorption spectroscopy (SEIRAS). In this poster current work and outlook on this topic will be presented.

Poster Presentations: Group B

In Situ Tracking of Surface Reactivity during Lead-Acid Battery Refurbishment via Chelation Treatment

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Lead-acid batteries (LABs) have been and continue to be one of the most widely used rechargeable batteries, making up of 70% of the worldwide secondary battery market. Because of their safety, performance, and low cost, LABs are widely used in the microgrid, photovoltaic and mobility sectors. However, LABs have a relatively short cycle life (≈300 cycles), resulting in increased replacement costs and a high environmental impact, producing extensive amounts of heavy metal wastes. The failure of the LAB mostly results from the hard sulfation, a surface phenomenon. When the LAB is operated under a partial state of charge, cycled at high rates, deeply discharged, or stored in the discharged state, large and irreversible PbSO4 crystals are formed, particularly at the negative electrode surface. This increases the cell resistance and reduces the redox active material available that leads to a decrease of the capacity of LAB and eventually to the failure of the battery. Removing large PbSO4 crystals using chelators is regarded as a promising approach to reverse the capacity loss of LABs. However, its evaluation requires a method to track the removal efficiency of PbSO4 crystals and the renewal of redox reactivity at the microscopic level. In my presentation, we will show an analytical methodology combining surface interrogation mode of scanning electrochemical microscopy (SI-SECM) and Raman spectroscopy to locally track surface redox reactivity during hard sulfation and test inorganic and organic chelation treatment protocols. SI-SECM was used to elucidate the mechanism and kinetics of sulfation and refurbishing procedures, while combined Raman/SI-SECM showed the non-localized nature of PbSO4 formation. Combining SECM imaging and coulometric experiments with spectroscopy and ex-situ characterizations, valuable insights into the rich chemistry of LABs were derived. Our experiments hence suggest exciting, novel, and environment-friendly directions for refurbishing LABs.

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

Zihao Qi (1), Charles Bell (1), Vicki Wysocki (1)
(1) The Ohio State University

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 monitor the chemical kinetics for the formation of complexes. These results will provide better insights on DNA repair, which will lead to further therapeutic development to treat various cancers.

Poster Presentations: Group B

Evaluation of multiple photosensitizers for singlet oxygen based isomer-resolved mass spectrometry of lipids.

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Reaction between singlet oxygen (SO) and C=C double bonds enable isomer-resolved mass spectrometry imaging (iMSI) and identification of lipid isomers. SO reacts with C=C bonds, converting lipids into lipid hydroperoxides (LOOH). Subsequent collision induced dissociation (CID) of LOOHs produces unique fragments corresponding to the C=C position. For the reaction to be successful, it is imperative to select a photosensitizer with long triplet state lifespan, high quantum yield, and a wavelength of absorption that may be accessed using accessible light sources. Herein, we explore and evaluate the photosensitizing capabilities of rose Bengal (RB), methylene blue MB), and zinc tetraporphyrin (ZnTPP) in the bulk analysis of lipid extracts in search of a potential universal photosensitizer for lipid isomeric differentiation. Methods Lipid extracts of wild type mice brain tissues were used for analysis. Aliquots were spiked with LPE 17:1 as an internal standard, the respective photosensitizer, and placed under white light for 10 minutes for SO formation. Bulk analysis was done by ESI on a QExactive HF-X mass spectrometer. LOOHs produced from lipids of interest were selected for MS/MS analysis. A standard solution of each photosensitizer was measured using a Ocean optics DH-200 UV-Vis spectrophotometer. Methanol, DI water, and toluene we used as solvent components in varying ratios for each photosensitizer. Preliminary data RB (Φ=0.75) is an effective photosensitizer for isomer-selective lipid imaging in positive mode. However, RB generates abundant peaks in negative mode, which interfere with MS measurements in the lipid region. On the other hand, MB (Φ=0.52), does not ionize in negative mode, but has a dominating peak in positive mode at m/z 284. In contrast, ZnTPP (Φ=0.83 in benzene) is poorly ionized in both modes. We compare the efficiency of these phososensitizers for generating LOOHs of lipids in bulk analysis of lipid extracts and use UV/vis spectrophotometry to identify the optimal excitation wavelength for each photosensitizer. The information obtained from these experiments will help assess structural candidates in the search for a universal photosensitizer and help uncover more insights into lipid metabolism during disease at the cellular level. Novelty Aspect Use of established photosensitizers for an innovative application of lipid positional isomer identification through the singlet oxygen reaction.

Structural Characterization of Redox-Active Polyoxovanadates Decorated with Different Ligands Using Ion Mobility Spectrometry

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(1) Purdue University, (2) University of Rochester

Structural characterization of metal nanoclusters is challenging especially considering clusters with ligands that contain smaller atoms. Traditionally nanoclusters are characterized using crystallography, which is limited to species that can be purified and crystallized. Ion mobility spectrometry (IMS) provides an opportunity to structurally characterize nanoclusters in the gas phase without inferences providing insights into subtle changes in structures that cannot be detected by other structural characterization methods. We have synthesized a series of polyoxovanadates (POV) with a V6O7 core for applications in energy production. The ligands are shown to enhance the solubility of POV while maintaining redox activity. We use IMS to examine the mobilities of the POV core and provide evidence supporting the existence of POV isomers. We also consider the accurate CCS values of the known structural parents and can infer the structures of the mixed ligand species.

Poster Presentations: Group B

Synthesis of atomically precise iron sulfide clusters and their mass spectrometric analysis

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Nanoclusters consist of a core of atoms that are usually protected with surface ligands, intermediate in size between single atoms and nanoparticles. Their high surface to volume ratio, unique electronic structure, and tunability makes them a great candidate as a model system for fundamental studies and application in catalysis, energy storage, and molecular electronics. In this study, we designed nanoclusters using atom-byatom substitution to replace one of the Fe atoms in the core of [Fe6S8L6]+/2+ cluster with other transition metals. Because the core determines the overall properties of the nanocluster, this is an effective method for tuning the electronic and magnetic properties of the cluster. We synthesized nanoclusters based on the iron sulfide and nickel sulfide cluster protected with triethylphosphine ligand (PEt3) and analyzed them using high resolution mass spectrometry. Mass spectrometric analysis showed that when FeCl2 is used as a metal precursor in the synthesis singly and doubly charged cationic species are formed [Fe6S8L6]+/2+(L=PEt3). Meanwhile, using NiCl2 as a metal precursor generates singly charged [Ni3S3HL5]+ cluster with the same synthetic procedure. We examined the substitution of Ni atom to the core of [Fe6S8L6]+/2+ clusters by using 1:1 molar ratio of NiCl2 and FeCl2. Mass spectrometric analysis revealed signals corresponding to [Fe5NiS8L6]+, [Fe4Ni2S8L6]+, [Fe5NiS8L5]2+, and [Fe4Ni2S8L5]2+ species indicating that [Fe6S8L6]+/2+ cluster undergoes atom-by-atom substitution. The core of the [Ni3S3HL5]+ cluster does not undergo substitution with Fe atoms. This work increases the range of atomically precise alloy nanoclusters that have potential applications in molecular electronics, spintronics, quantum computing, and energy storage.

Metabolomic and Lipidomic Profiling of Bacillus Using Two-Dimensional Tandem Mass Spectrometry

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Lipidomic and metabolomic profiles of sporulated and vegetative Bacillus subtilis and Bacillus thuringiensis from irradiated lysates were obtained using a quadrupole ion trap mass spectrometer modified to perform two-dimensional tandem mass spectrometry (2D MS/MS). The 2D MS/MS spectra were acquired using a 1.2 second 2D MS/MS scan of negative ions generated by nano-electrospray ionization of microwave irradiated spores to detect dipicolinic acid (DPA), as well as various lipids components. Aside from microwave irradiation to extract DPA and lipids from spores, sample preparation was minimal. The number of spores and vegetative cells used in the experiments was on the order of 108—109. Characteristic lipid profiles were observed for each Bacillus species which can be used to differentiate the two. Furthermore, the metabolic profiles strongly differentiate the sporulated and vegetative state within each species. Major features of the lipid profile observed for the vegetative state were phosphatidylglycerol (PG) lipids as well as fatty acids. Product ion spectra were extracted from the 2D MS/MS data to provide structural information of the fatty acids components of the various PG lipids. The study demonstrates the flexibility, speed, and informative power of metabolomic and lipidomic fingerprinting for the potential identification of spore-forming biological agents using 2D MS/MS with minimal sample preparation.

Poster Presentations: Group B

Direct analysis of host-pathogen interactions in Pseudomonas aeruginosa biofilm-infected wound tissue by comparative lipidomic profiling and imaging using DESI-MS

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The ability of Pseudomonas aeruginosa (PA) to form biofilms (bacterial aggregates protected by an extracellular polymeric matrix) contributes to chronicity of wound infections. The extent of biofilm progression and pathogenicity is largely dependent on interactions between the PA and the host tissue microenvironment, further impeding wound closure. Given this, drug discovery efforts may consider targeting host factors in addition to the pathogen itself. However, new analytical methods are needed to accurately assay both systems in their native conditions to provide a translationally relevant understanding of biofilm infection, diagnosis, and treatment. Traditional methods of biofilm analysis often involve significant and timely sample preparation followed by microscopy, genomic sequencing, immunoassays, analyte extraction, and/or MS (i.e., LC-MS, MALDI). Here we demonstrate the utility of DESI-MS to rapidly determine host-pathogen interactions by imaging and profiling the lipidomes of PA biofilms and associated host tissues directly from an immunocompetent preclinical porcine model with unprecedented time savings and simplicity. DESI-MS sampling (1 sec/sample) provided robust lipidomic data from i) PA biofilms on agar either exposed to wound fluid or not and ii) tissue cryosections on glass slides from PA-infected or native flora-infected porcine wounds. Using the mass spectral information, feature selection and dimensionality reduction approaches enabled the determination of diagnostic ions for treated and untreated samples, as well as across time. DESI imaging enabled observation of the spatiotemporal distribution of notable features, namely the appearance of quorum sensing compounds, rhamnolipids, and oxidized fatty acids. Findings were verified using MS/MS and/or multiple reaction monitoring (MRM). These analyses revealed a marked increase in sphingolipid metabolites in the biofilms and associated ceramide depletion in the host tissue. Additional insight is provided as to changes of bacterial membrane phospholipid composition, proliferation-associated compounds, and the abundance and diversity of quorum sensing signaling molecules produced by PA over time.

Chiral-Specific Optical Responses of Uniaxial Assemblies

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While the relationship between molecular chirality and circular dichroism has been studied for over a century and has since developed into a regular analytical technique, the relationship between molecular chirality and the optical response of the resulting uniaxial assembly is presently ill-understood. Uniaxial assemblies represent the next lowest symmetry below isotropic and are especially important in studies of interfacial interactions. Until recently, most efforts have focused on extract molecular information from assembly responses by minimizing observed contributions. Integrating ensemble symmetry and molecular chirality into a predictive framework for optical responses provides a cohesive explanation for previously-observed phenomena and opens an entire class of potential information gained with simple modifications to existing commercial instrumentation and analyses. In this study, we extend a mathematical framework originally developed for chiral-specific, coherent four-wave mixing processes to envelop chiral-specific absorption and fluorescence. Through this, we proposed relationships between observed optical responses and sample orientation. Circular dichroism absorbance spectra of naproxen crystals obtained on a commercial Jasco 1500 spectrometer have since corroborated mathematical predictions.

Poster Presentations: Group B

Two-Dimensional Tandem Mass Spectrometry for Mixture Analysis of Individual Compound Functional Group Identification

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The mixture analysis of individual compounds is usually performed with laborious sample clean-up, sample preparation and separation, which are time-consuming. Here, a novel method of quick mixture individual compounds' functional group identification/structural elucidation is presented. Two-Dimensional Tandem Mass Spectrometry (2D MS/MS) allows data-independent MS/MS scans over the mass range within a single scan. The excitation and ejection RF is ramped, and each m/z is fragmented with a linear relationship to time. The intrinsic separation and dissociation of ions based on precursor m/z inside the ion trap grants a highly efficient MS/MS analysis of each individual compound in a mixture within 1 sec. To aid the structural elucidation, online derivatization with the reactive ionization method (nESI, reactive DESI, and paper spray), which is further facilitated by reaction acceleration in microdroplets and thin films, was used to identify specific functional groups. This manipulation allows 1. Functional group information due to reactivity 2. Additional structural information of the original compound due to different fragmentation patterns after derivatization. Specifically, the pre- and post-derivatization 2D MS/MS spectra were normalized and subtracted from each other to reveal the changes (or the lack thereof) of individual compounds to the selected derivatization method. This resulted single final spectrum confirms the presence of a given chemical class (or classes) within a mixture and structural elucidation. Proof of concept experiments were successfully performed for the identification of primary and secondary amine compounds in an amine mixture using the Katritzky transamination reaction, amidation reaction of succinic anhydride, and alkylation reaction of benzylbromide as the derivatization methods. There are two specific goals following this identification of functional groups: 1. Improve the detection of low-intensity abused drug (i.e. cathione compounds via selective derivatization of aromatic aldehyde) in a complex biological matrix (i.e., urine) for forensic application, and 2. Develop an automated structural elucidation tool by using specific expert rules such as the shifts of precursor m/z, product ion m/z, neutral loss, and fragmentation pattern prediction.

Substituent influence on degradation pathways of conjugated radical cations

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π-conjugated organic materials are promising candidates for a wide variety of opto-electronic technologies, due to their tunable redox and optical properties. While the possibility of large-scale synthesis and processability give organic semiconductors an edge over certain inorganic counterparts, material stability issues remain a major hurdle, limiting the widespread adoption of these materials for commercial applications. The charged and/or radical species that are formed during electrochemical processes can be especially vulnerable to side reactions that degrade long-term functionality of organic materials. In this work, we focus on a group of thiophene-based conjugated molecules and investigate substituent influence on the stability of their electrochemically doped states. When oxidised into radical cations, characteristics of these molecules showed significant deviations from theoretical predictions. Using a combination of mass spectrometry, UV-visible spectroscopy and single crystal XRD analysis we identify two distinct degradation pathways for conjugated radical cations, involving functional group loss and sigma-dimerization. DFT calculations were used to predict the role of substituents in determining the degradation pathway, showing good agreement with experimental observations. These findings provide new insights into understanding and improving the stability of redox-active conjugated systems.

Poster Presentations: Group B

Biofuel-Specific Molecular Composition of Organic Aerosol in Biomass Burning Smoke

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Organic aerosol (OA) emitted from wildfires and prescribed forest and agricultural burning have diverse composition that undergo complex reactions and transformations in the atmosphere, leading to profound impacts on air quality, climate, and atmospheric chemistry. Emission characteristics are strongly dependent on regional vegetation, with different fuels resulting in substantially different OA component profiles. Understanding the emission profiles of these biomass fuels is fundamental in predicting their impact on atmospheric consequences of wild and prescribed fires. In this work, we characterize OA emissions of peat, sage grass, grass, and ponderosa pine burned in controlled laboratory environments. We utilize ultra-high-performance liquid chromatography coupled to a photodiode array detector and electrospray ionization high-resolution mass spectrometer (UPLC-PDA-ESI-HRMS) to investigate molecular characteristics of smoldering-phase emissions to model the contributions of these fuels in real-world burns. We showcase a method to determine the volatility profiles of the OA samples representative of studied fuel-types and their relationship to the light-absorbing properties of brown carbon (BrC) with respect to four optically based BrC classes (very weak, weak, moderate and strongly absorbing). This method of classification is practical for analyzing and comparing light absorbing OA samples and is applicable for both laboratory-based and real-world studies.

Real-time precision optical microsurgery (RPOMS) of single cells

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Spatially precise manipulation of subcellular targets is critical to understanding cellular functions. The traditional microsurgery methods for single cells require either microneedles or capillaries to pierce cell membranes. Such an approach is invasive and can only interact at a few sites in a cell. Another way is to use a confocal microscope to image cellular compositions and pinpoint femtosecond laser pulses to disrupt a selected target. Cellular organelles are heterogeneously distributed and are highly dynamic in live cells. Therefore, existing microsurgery methods cannot simultaneously detect multiple targets of interest and follow them in real time for precision optical perturbation. We developed a real-time precision optical microsurgery (RPOMS) platform to detect chemical compositions in live cells and simultaneously interact with these targets with laser pulses in real time. The RPOMS system is built upon a high-speed laser scanning microscope. During laser scanning, RPOMS will recognize a chemically specific optical response from molecular targets via multiphoton excitation fluorescence or coherent Raman scattering signals. The signals will then be used to activate an acousto-optic modulator (AOM), which will enable a high-energy laser pulse to only interact with the molecules of interest without affecting other components of the sample. This method is highly chemically specific, has submicron spatial accuracy, and has a nanosecond response time for automatic probing and disrupting of biomolecular activities in dynamic live cells. The entire decision-making process for feedback is automated and occurs within a single image pixel. A pulse-picking method is used to generate high-intensity laser pulses for microsurgery. We applied RPOMS to interact with different cellular organelles and studied the femtosecond-laser-microsurgery-induced cellular changes.

Poster Presentations: Group B

Molecular and Structural Characterization of Isomeric Compounds in Atmospheric Aerosols Using Ion Mobility – Mass Spectrometry

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Secondary organic aerosol (SOA) formed through multi-phase atmospheric chemistry makes up a large fraction of airborne particles. The chemical composition, molecular structures, and emission sources are complex and vary between different SOA samples, complicating their identification in complex mixtures. In this work, we utilize drift tube ion mobility spectrometry with quadrupole time-of-flight mass spectrometry detection for rapid gas-phase separation and multi-dimensional characterization of isomers in two sets of biogenic SOA samples (D-limonene, LSOA and **α**-pinene, PSOA). All samples were ionized using electrospray ionization (ESI) and acquired in both positive and negative ion modes. The IM-derived collision cross-sections in nitrogen gas (CCS) for isomer components in the samples were obtained using multi-field measurements. Novel ion multiplexing/high-resolution demultiplexing strategies were used to increase the sensitivity and augment mobility baseline resolution, which helped reveal several conformational and isomeric structures for the measured ions. For LSOA and PSOA samples, we report significant structural differences of the isomer structures complemented by theoretical calculations. The average CCS values for monomeric structures measured as [M+Na]+ ions are 1.2% higher than [M-H]- counterparts, meanwhile, dimeric and trimeric isomer structures in both SOA samples where 3.5% – 7% higher for [M-H]- than their [M+Na]+ ion structures, respectively. The results indicate that the structures of oligomeric ions coordinated Na+ ion are more compact than those of the corresponding deprotonated molecules. Meanwhile, deprotonated molecules have higher CCS values due to their slightly more elongated structures in the gas-phase. Therefore, DTCCSN2 values of isomers in SOA mixtures depend strongly on the mode of ionization in ESI. Additionally, PSOA monomers and dimers exhibit larger DTCCSN2 values (1 – 4% deviation) than their LSOA counterparts owing to more rigid ion structures in the gas-phase. Last, we investigated effects of direct photolysis on the chemical transformations of individual PSOA components. We use IM–MS to reveal structural changes associated with aerosol aging by photolysis. This study provides detailed molecular and structural descriptors for the detection and annotation of structural isomers in complex SOA mixtures.

Poster Presentations: Group B

Periodic Photobleaching with Structured Illumination for Diffusion Imaging

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The use of periodically structured illumination coupled with spatial Fourier-transform fluorescence recovery after photobleaching (FT-FRAP) was shown to support diffusivity mapping within segmented domains of arbitrary shape. Periodic "comb-bleach" patterning of the excitation beam during photobleaching encoded spatial maps of diffusion onto harmonic peaks in the spatial Fourier transform. Diffusion manifests as simple exponential decays of the spatial harmonics in the FT-domain, improving signal to noise and simplifying mathematical analysis. Image segmentation prior to Fourier transformation enables analysis of regions of arbitrary shape expected to exhibit constant diffusivity within a domain. Following proof-of-concept analyses based on simulations with known ground-truth maps, diffusion imaging by Fourier transform FRAP (FT-FRAP) was used to map spatially-resolved diffusion differences within phase-separated domains of model amorphous solid dispersion spin-cast thin films. Notably, multi-harmonic analysis by FT-FRAP was able to definitively discriminate and quantify the roles of internal diffusion and exchange to higher mobility interfacial layers in modeling the recovery kinetics within thin amorphous/amorphous phase separated domains, with interfacial diffusion playing a critical role in recovery. These results have direct implications for the design of amorphous systems for stable storage and efficacious delivery of therapeutic molecules.

Poster Presentations: Group B

A one-pot analytical pipeline for efficient and sensitive proteomic analysis of extracellular vesicle

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Extracellular vesicles (EVs) are important mediators in intercellular communication and play key roles in many physiological processes. Because of the involvement in several diseases, EVs from biofluids have been drawing more attention in the field of translational and clinical medicine. EV proteomics is particularly a promising tool in discovering potential biomarkers for disease diagnosis, monitoring, and therapeutics. However, the current workflow of mass spectrometry-based EV proteome analysis is not well compatible with clinical setting due to inefficient EV isolation methods and time-consuming sample preparation processes. Our group recently developed a rapid EV isolation technique called EV total recovery and purification (EVtrap) which applied chemical-modified magnetic beads and showed greater effectiveness compared to standard ultracentrifugation method. In order to further implement highly efficient and robust EV proteome analysis, this study established a one-pot analytical pipeline based on EVtrap beads to detect urinary EV proteome in a fast and sensitive manner. By incorporating on-bead lysis, digestion, and extraction, the one-pot pipeline avoided sample transfer steps and largely reduced the complexity of peptide preparation process for bottom-up proteomic analysis. Moreover, a shorter digestion time was practicable in this novel pipeline, which enables a whole EV proteome analysis to be completed within one day. In comparison with conventional workflow, one-pot pipeline was able to extract higher peptide amounts and identify similar numbers of unique protein in 1 mL of urine sample. Further evaluations revealed the feasibility of small sample amount in one-pot pipeline; ~800 unique EV proteins could be identified in only 20 µL of urine. Finally, we applied the one-pot pipeline to monitor potential biomarkers in urinary EVs of bladder cancer patients. A total of 3210 proteins including several known urinary EV biomarkers in bladder cancer were successfully identified in 53 urine samples using 15-min short gradient and directDIA . Compared to the control group, 37 significantly upregulated proteins and 27 significantly downregulated proteins were identified in patients. Taken altogether, our novel one-pot analytical pipeline demonstrated its power for routine and robust EV proteomics in the biomedical applications.

Poster Presentations: Group B

Chemical reactions in a molecular container: Dimerization in the Gas Phase

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We are exploring an approach for the study of reactions involving ions of the same initial polarity within an oppositely-charged molecular container. The process is a bridge between purely gas-phase reactions and condensed-phase reactions and might prove to be a rapid and specific means for the study of chemical reactivity. The idea is to inject sequentially two reactant ions of the same polarity into a large oppositely-charged ion that can serve as a reaction vessel. Dendrimers are polymers with a dendritic, treelike structure with cavities that can contain the injected reactants and have been used here to test this idea. Experiments were conducted on a hybrid triple quadrupole/linear ion trap tandem mass spectrometer QTRAP4000, AB Sciex, modified for ion-ion reactions. A dual pulsed nESI arrangement allowed for the sequential injection and subsequent reaction of negatively charged dendrimer reagent ions and positively charged analyte ions. Reactions were performed in the second quadrupole cell, Q2, applying an auxiliary frequency to the two containment lenses (IQ2and IQ3), located at either end of Q2, in order to trap both polarities of ions simultaneously. The product ions were then transferred to Q3 where mass-selective axial ejection (MSAE) was performed. The time frame of each step as necessary depending on ion abundances. A novel charge inversion process that involves the encapsulation of analytes into the cavities of a dendrimer to enable their reaction is being studied. In this work, we explore conditions that maximize the dimerization of two cations injected into highly charged dendrimer anions. The observation of a negatively charged dimer generated from the injection of two cations into a dendrimer indicates that they find each other and exit the complex as an anion. We demonstrate this phenomenon with various corticosteroids and sugars using anions derived from a half-generation polyamidoamine dendrimer (PAMAM 3.5G) as the reaction vessel. We also observe removal of the excess cation from an analyte ion and the transfer of an anion to the neutral analyte in a single ion/ion encounter. This work constitutes a proof-of-concept for a novel means for the study of the reactivity of mass-selected species that mimics the condensed phase.

Poster Presentations: Group B

Active Learning to Optimize Experimental and Modeling Parameters in Chemical Sciences

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Parameter optimization for experiments, instruments and models are essential in all of chemical sciences spanning different fields including analytical instrumentation to chemical synthesis and processes. Specifically, objectives (desired outputs) need optimization of parameters (inputs) that often take long hours and specific expertise to optimize. The current method of parameter optimization includes a lot of manual effort, in that scientists optimize parameters by large-scale brute force experimentation for randomly screening parameters, scientific intuition, extrapolating from existing knowledge and learning from iterative trails that is only manageable for small number of parameter optimizations (such as reaction condition optimization, instrument parameters for calibration, etc.). Several algorithms have been developed for automating this iterative learning process of learning, a form of active learning, such as evolutionary algorithms (EA) and Bayesian Optimization (BO) that help expedite and automate chemical processes. Here, we employed examples of both EA and BO algorithms to optimize parameters through model building from hyperparameter optimizations used for machine learning to finding conditions for experiments in chemical reactions. Specifically, we compared Paddy, the EA developed in our group with BO algorithms with several simulated examples where the underlying relationship of the objective was unknown towards iteratively optimizing parameters. In addition, we also optimize multiple objectives (yield and production rate) for a chemical reaction in a continuous flow reactor iteratively by changing time, temperature, and mole fraction of pyridine suggested by the algorithm. We were successfully able to optimize the objectives of the reaction in 10 iterations in addition to developing user friendly Jupyter notebooks. The initial set of conditions was randomly chosen by a human chemist, but all subsequent rounds of conditions were selected by the model. In addition, the optimal conditions were found through a response function with a sigmoid penalty and the data was visualized using a pareto plot to define the boundary of possible optimal conditions. Several other examples are employed where BO is compared with EA (Paddy) that provides a larger sampling space of parameters towards solving such problems. With these simulations, we show that EA and BO identify optimal conditions more efficiently than random trails, thereby saving resources and provides a framework to optimize parameters for any objective in the chemical sciences.

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