Biomaterial Interfaces
Room 321 - Session BI3-TuM

Characterization of Biological and Biomaterials Surfaces
Moderator: Roberto Eguiluz, University of California Merced

11:15am **BI3-TuM-14 ToF-SIMS Analysis of Tissue Microenvironments and Tumor Growth Progression**, ***Lara Gamble***, *Daniel Graham,* University of Washington

Tissue microenvironments are a complex ecosystem of cell and non-cellular (e.g. chemical signaling molecules). The regulation of cell growth, metastatic potential, and drug resistances are associated with the microenvironments around solid tumors. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is a sub-micron spatial resolution imaging mass spectrometry technique used for chemical imaging. Here a combination of ToF-SIMS and H&E staining are used to analyze mouse pancreatic tissue slices by imaging tumors in a *pIns-MycER^{TAM}/p53$^{-/-}$* mouse model. TOF-SIMS was performed with an ION-TOF TOF.SIMS 5-100 instrument (ION-TOF GmbH, Münster, Germany) equipped with a liquid metal ion gun (LMIG) for analysis and an electron flood gun for charge neutralization. Analyses of the *pIns-MycER^{TAM};p53$^{-/-}$* tumor microenvironment has identified and mapped molecules that play key roles in tumor metabolism and growth including nucleic acids, organic acids and lipids, as well as inorganic ions. Evidence is provided of significant metabolic change occurring within *Myc*-driven β cell tumors and surrounding stromal region over a 12-day period and demonstrate that TOF-SIMS imaging is a useful tool for spatial characterization of metabolite changes in the *Myc* model. Analyses provide evidence that significant metabolic changes occur in the tumor microenvironment in response to *Myc* activation and that the metabolic profiles of the tumor and the stroma differ dramatically.

11:30am **BI3-TuM-15 Molecular Analysis of Small Extracellular Vesicles by Nanoprojectile Secondary Ion Mass Spectrometry**, ***Pierre Hirchenhahn***, *Stanislav Verkhoturov, Victoria Cura, Ravi Jada,* Texas A&M University*; Thanh Qui Nguyen, Daheui Choi,* Mayo Clinic*; Michael J. Eller,* University of Mississippi*; Alexander Revzin,* Mayo Clinic*; Emile A. Schweikert,* Texas A&M University

Small extracellular vesicles (SEVs) are biological objects of 30 to 150 nm. They serve as communication line between cells and their environment through the transport of lipids, proteins and genetic material. Because they carry tissue- and health-status-specific biomarkers, SEVs are highly promising candidates for disease diagnosis and monitoring. However, their strong chemical heterogeneity makes their characterization particularly challenging. Current analytical techniques generally provide only partial or averaged information, preventing a comprehensive understanding of SEVs molecular diversity. In this context, Nanoprojectile Secondary Ion Mass Spectrometry (NP-SIMS) appears as a powerful solution. Unlike conventional SIMS approaches, NP-SIMS operates in an event-by-event mode, where the analyte is impacted by a single nanoprojectile at a time. The nanoprojectiles used are Au_{400}^{4+} clusters accelerated to 100 kV, generating craters of only 10–20 nm in diameter. The secondary ions emitted from each individual impact are mass analyzed and recorded separately, providing unprecedented molecular lateral resolution and sensitivity. A critical challenge for NP-SIMS investigations is the preparation of suitable capture surfaces ensuring both high EV density and minimal aggregation, so that each nanoprojectile impacts only a single vesicle. To address this issue, plasma-cleaned silicon wafers were functionalized with successive layers of (3-Aminopropyl)triethoxysilane (APTES) and glutaraldehyde prior to EV incubation. The different preparation steps and resulting surface chemistry were assessed through a combination of NP-SIMS, X-ray photoelectron spectroscopy (XPS), and scanning electron microscopy (SEM). Finally, the validity of the developed preparation protocol was demonstrated using hepatic extracellular vesicles.

This work was supported by NIH grant number 5R01DK134661.

11:45am **BI3-TuM-16 Nanoscale Characterization of Bio-Nanomaterials Using Nano-Projectile Secondary Ion Mass Spectrometry**, ***Christelle Guillermier***, Bienne Technology*; Michael Eller,* University of Mississippi*; Stanislav Verkhoturov,* Texas A&M University*; Richard Rickman,* Bienne Technology*; Emile Schweikert,* Texas A&M University

Advances in nanoparticle biomaterial engineering have made accurate characterization of these systems increasingly important. Many modern nanoparticle designs are multifunctional, resulting in complex and often heterogeneous composition, including hybrid and core/shell architectures, particles functionalized with multiple ligands, asymmetric particles, sensing platforms, and targeted therapeutic carriers each presenting distinct analytical challenges.

To address these challenges, we present Nano Projectile Secondary Ion Mass Spectrometry (NP-SIMS), a variant of conventional SIMS that enables nanoscale molecular analysis of individual nanoassemblies. In contrast to conventional SIMS, which relies on continuous ion beams with spatial resolutions typically limited to ~30 nm, NP-SIMS employs ~2 nm gold nanoprojectiles delivered as isolated impacts separated in both time and space. A time-of-flight (ToF) mass spectrum is acquired from each individual impact event. Each nanoprojectile probes a nanovolume approximately 10 nm in diameter, allowing nanoassemblies to be analyzed one-by-one rather than as ensemble averages. This event-by-event acquisition approach enables the direct identification of colocalized molecular species within complex nanoassemblies.

Here, we present several examples illustrating the application of NP-SIMS to the characterization of complex biomaterial nanoassemblies. First, NP-SIMS was used to analyze extracellular vesicle (EV) population heterogeneity at single-EV resolution and to distinguish hepatocyte-derived EVs from liver cancer EVs based on differences in surface marker expression. Second, we demonstrate the determination of DNA ligand loading and ligand density on DNA functionalized metal nanoparticles, specifically anisotropic gold nanostars and isotropic gold nanospheres with comparable surface areas. Third, NP-SIMS was applied to the analysis of a mixture of nanoparticles containing identical metal cores (3–5 nm in diameter) but functionalized with ligands of different chain lengths (decanethiol, tetradecanethiol, and hexadecanethiol). Using NP-SIMS, we determined the relative abundance of the three nanoparticle populations on the surface.

These examples demonstrate the capability of NP-SIMS to characterize molecular composition, heterogeneity, and surface functionalization within complex nanoscale biomaterial systems at the level of individual nanoassemblies, providing analytical information inaccessible through ensemble-averaged measurements.

12:00pm **BI3-TuM-17 Interactions of Divalent Metal Cations with Phosphate Headgroups in Monolayers at the Air/Water Interface**, ***Sadia Afroz***, *Paul S. Cremer,* Pennsylvania State University

The binding of divalent metal cations to negatively charged monolayers in aqueous solutions is a complex process that depends on the supramolecular arrangement and spacing of the headgroups at the air/water interface. Previous literature showed that binding of Mg^{2+} and Ca^{2+} to the PIP(4,5)P_2 head groups has significant impact on interactions between PIP(4,5)P_2 and protein by changing the orientation of the head group and ordering of the acyl chain. Moreover, Ca^{2+}forms contact ion pairs with PIP(4,5)P_2 head groups, whereas Mg^{2+}preferentially forms solvent-shared ion pairs. Herein, Sum Frequency Generation (SFG) vibrational spectroscopy was employed to probe the water structure and mode of ion binding with two different PA species, 1,2-dilauroyl-sn-glycero-3-phosphate (DLPA) and 1-stearoyl-2-hydroxy-sn-glycero-3-phosphate (LPA). It was found that increasing the PA charge density and fraction deprotonation increased the binding affinity in both cases, but LPA showed stronger sensitivity than DLPA. These data also suggested that differences in the binding behaviour between $Ca^{2+}$and Mg^{2+}arose from the molecular-level details of the binding mechanism. The spacing of the headgroup was important and the phosphate moieties could be closer together with LPA (1 tail) compared to DLPA (2 tail). This study provides important insights into the mechanisms underlying the binding of divalent cations to PA, which may have implications for understanding cellular signaling.

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