Biomaterial Interfaces Division
Room 101B - Session BI+AS+IPF+MN-MoA

Advanced Imaging and Structure Determination of Biomaterials Research

Moderators: Dan Graham, University of Washington, Axel Rosenhahn, Ruhr-University Bochum, Germany

1:20pm **BI+AS+IPF+MN-MoA1 NMR Relaxometry as a Medical Diagnostic*, Michael J. Cima,*** Massachusetts Institute of Technology **INVITED**

This talk will describe the diagnostic capabilities of magnetic resonance imaging (MRI) when brought to the patient bedside. Rather than imaging, NMR can be used for important chemical/physiologic diagnostic endpoints. Two will be discussed here; quantifying fluid overload and measurement of hypoxia within tumors. Assessment of intra- and extra-vascular volume is integral in managing patients with heart, liver, and kidney disease as volume status is closely linked to mortality. Commonly used determinants of volume status, such as physical exam and ultrasonography, lack sensitivity and specificity and require expertise in clinical practice. This talk reports on nuclear magnetic resonance (NMR) methods to a portable and clinically useful device. A clinical study with hemodialysis patients and age-matched healthy controls was performed at MGH. The T2 relaxation times of study participants' legs were quantified at multiple time points with both a 1.5T clinical MRI scanner and a custom 0.27T single-voxel MR sensor. The results showed that first sign of fluid overload is an increase in the relative fraction of extracellular fluid in the muscle. The relaxation time of the extracellular fluid in the muscle eventually increases after more fluid is accumulated. Importantly, these MR findings occur before signs of edema are detectable on physical exam. Solid tumors are often hypoxic and characterized by an extreme lack of oxygen. Tumor hypoxia imparts significant negative outcomes for patients but is highly variable within cancer types and patient populations. Many of these poor clinical outcomes can be tied to hypoxic-induced radiotherapy resistance. Resistance to radiotherapy in hypoxic regions can be overcome by increasing the dose delivered but exposure limitations of healthy tissue and organs must be considered. The lack of a viable quantitative clinical oxygen measurement method prevents safe dose escalation in these patient populations. Here we report on a silicone-based quantitative oxygen sensor. The MRI contrast of this material depends on dissolved oxygen. Thus, the material functions as a first of its kind solid-state contrast agent. The sensor leverages the existing MRI hardware, which is part of the current clinical work flow, to map tumor oxygen content. This information can then be integrated into the dose planning process clinicians currently conduct to selectively and safely boost dose to low oxygen tumor subvolumes. This sensor is approved by the institutional review board at Dana Farber Cancer Center for a clinical trial in patients locally advanced cervical cancer.

2:00pm **BI+AS+IPF+MN-MoA3 Direct Observation of Cell Signaling Proteins Interacting with a Model Cell Membrane by Sum Frequency Generation Vibrational Spectroscopy***, T.W. Golbek,* Oregon State University*; T. Weidner,* Aarhus University, Denmark*; C.P. Johnson, **Joe Baio**,* Oregon State University

Proteins that contain C2 domains are involved in a variety of biological processes including encoding of sound, cell signaling, and cell membrane repair. Of particular importance is the interface activity of the C-terminal C2F domain of otoferlin due to the pathological mutations known to significantly disrupt the protein's lipid membrane interface binding activity, resulting in hearing loss. Therefore, there is a critical need to define the geometry and positions of functionally important sites and structures at the otoferlin-lipid membrane interface. Here we describe the first *in situ* probe of the protein structure of otoferlin's C2F domain interacting with a cell membrane surface. To identify this protein's structure at the lipid interface we applied sum frequency generation (SFG) vibrational spectroscopy and coupled it with simulated SFG spectra to observe and quantify the otoferlin C2F domain interacting with model lipid membranes. A model cell membrane was built with equal amounts of phosphoserine (PS) and phosphocholine (PC). SFG studies that examined the ordering of the lipids that make up the model membrane, demonstrate that lipid fusion occurs after docking of the otoferlin C2F domain via the observation of a 62% increase in amplitude from the SFG signal near 2075 cm^{-1} assigned to specific groups within the model membrane. This increase is related to lipid ordering caused by the docking interaction of the otoferlin C2F domain. SFG spectra taken from the amide I region contain peaks near 1621 cm^{-1} and 1672 cm^{-1} related to the C2F domains beta-sandwich secondary structure, thus, indicating that the domain binds in a specific orientation. By mapping the simulated SFG spectra to the experimentally collect SFG spectra, we found the C2F domain of otoferlin orients 32° normal to the lipid surface. This information allows us to map what portion of the domain directly interacts with the lipid membrane. Furthermore, we show first experimental view of any C2 domain of otoferlin docked at the membrane interface, thereby, validating SFG as a method to probe C2 domain-membrane interfaces.

2:20pm **BI+AS+IPF+MN-MoA4 Vibrational Sum-frequency Scattering Spectroscopy for the Characterization of Protein Fiber Structures and their Surface Interactions in Biological Environments*, Patrik K. Johansson,*** *D.G. Castner,* University of Washington

Biological processes are typically regulated by interactions at the interface of 3D structures, such as the membrane of cells or protein fiber surfaces. Collagen (the most common protein in mammals) forms large fibers that are responsible for the structural integrity of tissues. The structure, organization and interactions of these fibers are furthermore important for the survival, communication, migration, and proliferation of cells.

Investigating protein fiber interactions is challenging, particularly under biological conditions where the fibers exist in a 3D aqueous environment. Many techniques cannot interrogate interfaces buried in the bulk of a solvent and therefore require 2D surface models, while others need extensive purification and sample preparation. These approaches may not capture all key characteristics of the fiber surface structure and interactions in the real sample. However, vibrational sum-frequency scattering (SFS) spectroscopy, with inherent contrast for local molecular ordering, can be utilized towards these important goals.

As a first demonstration, we have applied SFS to protein fibers in aqueous environments, self-assembled from collagen type I. We detected signals from the amide I band and the N-H stretching vibrations, both of which are related to the specific protein backbone structure. Signals from the C-H stretching and bending vibrations were also identified, which are more associated with the side-chains in the fibers. The angular scattering patterns for the backbone (amide I) and side-chain (C-H stretches and bends) signals are different, making the spectra dependent on the angle of detection. While the backbone signals are dominant in the phase-matched direction, the side-chain signals remain high also at large scattering angles. Distinctions in the organizational symmetry and the relative fiber surface contribution to the overall signal are hypothesized as reasons for this observation.

Finally, we are investigating the impact of changes to the environment (e.g. ionic strength, pH, surfactants) on the shape of spectra and scattering patterns for the detected SFS signals. This could yield new insights to the structure and dynamics of collagen fibers in biological settings. The relevance of such investigations is enhanced by the fact that detection of vibrations from the surrounding molecules is a direct observation of their interactions with the collagen fiber surface, which thus can be correlated with the fiber structure. The relative orientations for the detected groups can also be obtained via vibrational SFS polarization analysis, for a deeper understanding of biomolecular interactions in biological processes.

2:40pm **BI+AS+IPF+MN-MoA5 How Proteins Grow Calcium Carbonates – The Mechanism of Vaterite Bioprecipitation Studied at the Molecular Level by Sum Frequency Generation Spectroscopy***, H. Lu,* Max Planck Institute for Polymer Research, Germany*; S. Roeters,* Aarhus University, Denmark*; H. Lutz, M. Hood, A. Schäfer,* Max Planck Institute for Polymer Research, Germany*; R. Muñoz-Espí,* Universidad de Valencia, Spain*; M. Bonn,* Max Planck Institute for Polymer Research, Germany*; **Tobias Weidner**,* Aarhus University, Denmark

Proteins can act as Nature's engineers at interfaces and manipulate hard tissue growths. Specialized peptides can bind and release specific mineral facets and grow the intricate mineral morphologies found in diatom cell walls, mollusk nacre, but also human teeth and bone. Taking clues from Nature we aim at understanding the mineralization processes at the molecular level and to develop design rules for biogenic nanophase materials. Mineral proteins control the biogenesis of $CaCO_3$ by selectively triggering the growth of calcite, aragonite or vaterite phases. The templating of $CaCO_3$ by proteins must occur predominantly at the protein/$CaCO_3$ interface. Surprisingly, molecular-level insights into the interface during active mineralization have been lacking. Here, we investigate the role of peptide folding and structural flexibility on the mineralization of $CaCO_3$. We discuss the mineral activity of amphiphilic

peptides based on glutamic acid and leucine with *ß*-sheet and *α*-helical secondary structures. While both sequences lead to vaterite structures, the *ß*sheets yield free-standing vaterite nanosheet with superior stability and purity. Surface-specific spectroscopy studies and molecular dynamics simulations reveal that the interaction of calcium ions with the peptide monolayer restructures both the peptide backbone and side chains. This restructuring enables effective templating of vaterite by mimicry of the vaterite (001) crystal plane. The approach is universally applicable to mineral peptide engineering. We will discuss how analogous peptide designs can be used to steer the growth not only of calcium carbonates but also calcium oxalates.

3:00pm **BI+AS+IPF+MN-MoA6 ToF-SIMS Imaging of Chemical Modifications in Topographically Challenging Materials***, **Michael Taylor**, D.J. Graham, L.J. Gamble,* University of Washington

Three-dimensional (3D) porous materials are applied in a variety of areas within materials science[1]. Pores in catalysts provide a high surface reaction area, pores in biofilters facilitate fluid movement for biomolecule capture, and pores in tissue engineered constructs allow for cellular ingress and vascularization. These applications require surface modifications to add specific functionality to their surfaces. The successful functionality of these materials is related to the ability of these modifications to reach all surfaces of the pores. However, it is challenging to characterize these complicated materials and verify the presence and distribution of these surface modifications. Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) is a powerful label-free surface analysis tool that can be used to image the molecular composition of cells, tissues and polymers. Porous 3D materials however, are non-ideal for ToF-SIMS analysis as the technique is highly surface-sensitive, topography on the order of microns can inhibit the ability to produce secondary ions related to surface modifications. To solve this problem we have developed a methodology for filling voids in porous materials to produce a surface where ToF-SIMS imaging may be performed. A embedding process for porous materials with poly(vinyl alcohol)(PVA) is detailed followed by freezing and cryo-sectioning to expose the modified scaffold interior. Here, we demonstrate the versatility of this method by high spatial resolution 3D imaging of a number of surface modifications in PCL poly(caprolactone) scaffolds[2]. Characterisation of fluorocarbon (FC) films deposited using octofluoropropane (C3F8) plasma enhanced chemical vapor deposition (PECVD) will be demonstrated, showing that increased treatment times deposits uniform coatings while shorter treatment results in a gradient distribution of FC throughout the PCL scaffold. Additionally we show data on imaging immobilized/adsorbed proteins within PCL scaffolds. Using this methodology we demonstrate that high spatial resolution label-free 3D imaging of chemical modifications in materials with complex geometries is now possible with ToF-SIMS.

Refs:

(1) Yang, X.-Y.; Chen, L.-H.; Li, Y.; Rooke, J. C.; Sanchez, C.; Su, B.-L. Hierarchically Porous Materials: Synthesis Strategies and Structure Design. *Chem. Soc. Rev.***2017**, *46* (2), 481–558 DOI: 10.1039/C6CS00829A.

(2) Taylor, M. J.; Aitchison, H.; Hawker, M. J.; Mann, M. N.; Fisher, E. R.; Graham, D. J.; Gamble, L. J. Time of Flight Secondary Ion Mass Spectrometry—A Method to Evaluate Plasma-Modified Three-Dimensional Scaffold Chemistry. *Biointerphases***2018**, *13* (3), 03B415 DOI: 10.1116/1.5023005.

3:40pm **BI+AS+IPF+MN-MoA8 Imaging Plant and Plant Growth-Promoting Bacteria Interactions Using Time-of-Flight Secondary Ion Mass Spectrometry***, **Xiao-Ying Yu**, R. Komorek, Z.H. Zhu, C.J. Jansson,* Pacific Northwest National Laboratory

We present the first imaging and spectra results of plant root interactions with plant growth-promoting bacteria (PGPB) using time-of-flight secondary ion mass spectrometry (ToF-SIMS), showing the successful application of delayed image extraction to study plant biology. Compared to MALDI (Matrix Assisted Laser Desorption Ionization), an imaging mass spectrometry technique widely used in plant studies,[1] SIMS is less destructive and provides submicrometer spatial mapping of molecular species of importance in metabolic processes. *Brachypodium distachyon* (Brachypodium), a genomics model for bioenergy and native grasses, is used due to its small diploid genome, close phylogenetic links to other grass species, relative ease of genetic transformation, short life cycle, small stature, and simple growth requirements.[2] Plant growth-promoting bacteria (PGPB) such as *Pseudomonas* and *Arthrobacter* were introduced to Brachypodium roots prior to analysis, and their potential effect on root extrusion was studied using ToF-SIMS imaging. Specifically, delayed image extraction was used in data acquisition. This approach was chosen to obtain high mass and high spatial resolutions.[3] Excellent SIMS imaging gives topographical description of the root surface with and without PGPB interactions. Distinctive characteristic peaks are observed, indicating compositional changes with and without PGPB introduction to the root surface beside visible surface morphological variations. Our initial results demonstrate that ToF-SIMS is a promising imaging mass spectrometry tool to study plant biology and root-microbe interactions and provide molecular-level insight at the biointerface with high spatial resolution.

References:

[1] D Sturtevant *et al.*, Three-dimensional visualization of membrane phospholipid distributions in Arabidopsis thaliana seeds: A spatial perspective of molecular heterogeneity, Biochimica et Biophysica Acta (2017),**1862**(2)*,* 268-81.

[2] T Girin *et al.*, Brachypodium: a promising hub between model species and cereals, J. Experimental Botany (2014),**65**(19)*,* 5683-96*.*

[3] QP Vanbellingen *et al.*, Time-of-flight secondary ion mass spectrometry imaging of biological samples with delayed extraction for high mass and high spatial resolutions, Rapid Comm. Mass Spectrom. (2015), **29** (13), 1187-95.

4:00pm **BI+AS+IPF+MN-MoA9 Imaging of Cells and Tissues with Helium Ion Microscopy***, J.A. Notte, D. Wei, **Chuong Huynh**,* Carl Zeiss Microscopy, LLC

Both optical and electron microscopy are well established techniques in the life sciences with established protocols for imaging and sample preparation. However the newly developed helium ion microscope has some unique advantages, and is gaining a reputation for providing insightful, easy to interpret images over a wide range of biological samples and bio-materials. This presentation serves as both an introduction to this novel technique and a review of recent results.

Because helium ions do not suffer appreciably from diffraction effects, they can be focused to a sub-nanometer probe, providing nanometer scale image resolution with a depth of focus that is well suited to complex surfaces and structures. As helium ions interact with the sample, they provide an abundance of secondary electrons that convey surface-specific and topographical information. Distinctly different from the conventional (gallium) focused ion beams, helium ions do not significantly damage the sample from the sputtering process. And importantly, helium ion microscopy is not affected by charging artifacts when imaging insulating materials, even glass slides, so there is no need for metal over-coating which would otherwise obscure finer details.

Example images will include a pancreatic cell membrane showing the pores and cilia present on their natural surfaces. Other examples will show the complex structure of the principal cell and intercalated cells of the collecting duct of a rat kidney. Other imaging results from diverse fields include stony corals, collagen networks, bone minerals, stereocilia, otoconia, actin filaments, and cryptococcus neoformans. False colorized images of the multi-ciliated epithelial trachea of an adult mouse and T4-phages will also be presented. Finally, new results will be shown from the SIMS spectrometer which provides elemental and isotopic information, and can be the basis for true colorizaton.

In this talk, an emphasis will be placed on the physics principles that enable these imaging results. The selected examples serve to demonstrate the breadth of results that can be attained with this relatively new technique.

4:20pm **BI+AS+IPF+MN-MoA10 Quantitative Analysis of Electrolytes in Microliter-size Blood Drops Congealed via HemaDrop™ using Ion Beam Analysis and SIMNRA***, H. Thinakaran, S.R. Narayan, J.M. Day, **Nicole Herbots**, F.J. Ark, B. Wilkens, M. Mangus, R.J. Culbertson,* Arizona State University

Accurate analysis of microliter blood samples can improve medical testing and forensics. Most critically ill patients suffer from hospital-acquired anemia due to the large volume currently required for blood diagnostic tests: 7 mL per vial.

Prior attempts by Theranos to analyze microliter-sized blood droplets in liquid form exhibit systematic errors greater than 10%, higher than the acceptable medical threshold.

This research investigates the accuracy of Ion Beam Analysis (IBA) performed on microliter-sized blood droplets congealed into Homogenous Thin Solid Films (HTSFs) using HemaDrop™, a new patent-pending technique using hyper-hydrophilic coatings to condense fluids into a uniform solid state with a smooth surface.

Prior to IBA analysis, the solidification of blood droplets into HTSF's is observed with optical microscopy and compared to conventional Dried Blood Spots (DBS). DBS exhibit phase separation between platelets and serum, with non-uniform, rough surfaces. Conversely, blood droplets solidified on HemaDrop™-coated surfaces are uniform and smooth, with little phase separation.

Next, quantitative compositional analysis using IBA is performed on µL blood drops solidified on HemaDrop™ coatings and is compared to results on DBS. HTSFs congealed on HemaDrop™-coated surfaces yield well-defined 2 MeV RBS spectra where individual species and electrolytes (C, N, O, Na, K, Ca, Cl, Fe) can be identified, while none can be distinguished on DBS.

The damage curve method [1] extracts elemental composition while accounting for possible IBA damage. Several consecutive spectra are taken on the sample, and RBS yields are interpolated to their original concentrations.

IBA simulations with the software SIMNRA enable comparison between RBS data and simulations, resulting in elemental composition accurate within 1%. Blood electrolyte compositions via SIMNRA are obtained on successive IBA spectra taken on different areas of the thin solid films and on different HTSFs congealed from the same blood. Relative error analysis between different HTSF samples establishes whether reproducibility within 10% can be achieved.

HemaDrop™ reliably creates stable, uniform, thin solid films to measure blood composition from µL-volume drops based on comparative IBA results and optical observations. Measurements of elemental composition of HTSF of blood samples are accurate and reproducible. HemaDrop allows for analysis in vacuo from µL of blood, greatly expanding the range of techniques that can be applied to identify elements and molecules (e.g., antibiotics, proteins).

[1] *Int & US Patent Pending, 2016, 2017

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