## Biological, Organic, and Soft Materials Focus Topic
## Room: 201 - Session BO+AS+BI-WeA

### Advances in Surface Analytical Methods for Organic and Biological Interfaces
**Moderator:** D.W. Grainger, University of Utah

1:40pm **BO+AS+BI-WeA1 In-situ Broadband Sum-Frequency Spectroscopy of Biomolecules at Interfaces**, ***P. Koelsch***, *V. Kurz, R. Schmidt,* University of Heidelberg, Germany, *C.L. Howell,* University of Maine, *M. Grunze*, University Heidelberg, Germany

Sum-frequency generation (SFG) spectroscopy has been employed to characterize a variety of biointerphases in extended spectral regions in aqueous environment. This contribution summarizes our recent SFG studies on DNA films, extracellular matrix compounds, switchable self assembled monolayers, and other biological molecules. Molecular level details revealed in these studies show that SFG offers the prospect of characterizing conformation, orientation and ordering of biological molecules at interfaces in situ. The technique of SFG spectroscopy is inherently surface specific having submonolayer resolution. Being an all-optical technique it can be operated in aqueous environment and even buried interfaces can be assessed. However, beside the obvious potential impact of SFG spectroscopy, to date, most studies of biological systems have only been performed in the CH and OH stretching vibration regions. This is related to the difficulties in generating tunable high energy light pulses with table top laser systems at the biologically relevant lower wavenumber region (amide and fingerprint) to gain SFG spectra with reasonable signal to noise ratios. In this contribution we show, that our broadband femtosecond SFG spectrometer provides spectral data in the amide I and fingerprint region in air and aqueous environment on a daily bases which opens the opportunity to characterize in situ orientation and conformation of a wider class of more complex biomolecules. We will summarize these biologically related SFG results and demonstrate the potential impact of this technique, also to introduce SFG spectroscopy as another method for examining biofilms ex situ and in situ.

2:00pm **BO+AS+BI-WeA2 Secondary Protein Structures in Barnacle Cement**, ***D.E. Barlow***, Nova Research, *G. Dickinson, B. Orihuela, D. Rittschof,* Duke University Marine Laboratory, *K.J. Wahl*, U.S. Naval Research Laboratory

Understanding the chemistry of barnacle adhesion is of great interest in the areas of marine biofouling prevention and materials science of adhesives. While most work on the chemistry of barnacle adhesion to date has focused on identifying the protein composition of barnacle cement, relatively little has been done to directly characterize structure of barnacle cement proteins in their native states. Such studies should provide further insight into relationships between chemical structure and adhesion, as well as the types of biochemical mechanisms that may play roles in barnacle cement curing. We have used atomic force microscopy (AFM), circular dichroism (CD), and Fourier transform infrared (FTIR) spectroscopy to characterize cements deposited on quartz and $CaF_2$ substrates in seawater by barnacles (Amphibalanus amphitrite) transferred from silicone release panels. AFM imaging consistently shows that secondary cement residues left on the substrates are fibrillar. Circular dichroism spectra of cement residues on quartz showed negative peaks centered near 225 nm and positive peaks at about 195 nm, indicating that the barnacle cements are primarily helical in structure, but also contain some β-sheet components. This is further confirmed by transmission FTIR of cement residues on $CaF_2$, for which the amide III band is found to be composed of a broad band centered ~ 1650 $cm^{-1}$ consistent with α-helical structures, and components near 1685 and 1630 $cm^{-1}$ consistent with β-sheet structures. These results suggest that the fibrillar structures are predominantly helical in structure, in contrast with fibrillar structures like amyloids that exhibit primarily β-sheet conformations.

2:20pm **BO+AS+BI-WeA3 Analysis of Biosamples with Imaging TOF-SIMS**, ***H. Nygren***, *P. Malmberg*, University of Gothenburg, Sweden **INVITED**

Secondary ion mass spectrometry (SIMS) is based on the acceleration of primary ions onto a target. Secondary electrons, neutrals and ions are emitted from the target reflecting its chemical composition. SIMS is unique in its ability to detect simultaneously several target molecules in compound samples and to image their localization at subcellular resolution. The aim of the project is to develop the technology of imaging TOF-SIMS as a tool in biomedical research for analysis and localization of relevant target molecules e.g. inorganic ions, lipids, drugs, carbohydrates and proteins in the same sample. Recent research in TOF-SIMS has shown the distribution of a wide variety of compounds in biological tissue. The current issues in TOF-SIMS analysis are the data interpretation and identification of all new peaks detected with this technique. Until 2004, only a few high mass peaks of biological origin were identified, which leaves us in a situation where much of the interpretation of spectra remains to be done. The aim of this presentation is to define analyte molecules by mass spectra obtained using new primary ion sources with unique fragmentation properties. The possibility to detect proteins is of special interest. It is also an aim of the project to educate students in the analysis of cells and tissue with a high technology method as the research group is presently purchasing our own TOF-SIMS instrument.

3:00pm **BO+AS+BI-WeA5 A Laser Desorption Vacuum Ultraviolet Postionization Imaging Mass Spectrometer for Biological Sample Analysis**, *J.F. Moore*, MassThink LLC, *A. Akhmetov, G.L. Gasper,* University of Illinois at Chicago, *R. Carlson,* Montana State University, *M. Blaze*, ***L. Hanley***, University of Illinois at Chicago

Creating images with mass spectrometry (MS) at <10 micron scale resolution is a substantial challenge due to diffusion effects and low signal levels. A new instrument is described for laser desorption and in-source postionization (LDPI) imaging mass spectrometry with enhanced performance over the previous non-imaging instrument.[1] A significant fraction of the desorbed molecules are intercepted by a vacuum ultraviolet (VUV) postionizing laser, then extracted into the time-of-flight (TOF) mass analyzer. Postionization provides a higher and more consistent sensitivity than matrix-assisted laser desorption/ionization for certain analytes, thereby enhancing imaging MS on this small length scale. The instrument utilizes 349 nm laser desorption with 5 micron minimum spot size, 157 nm laser postionization and will operate at ~200 Hz. Samples are analyzed in microprobe mode and are imaged by rastering the x-y stage. The ion source also incorporates digital optical imaging of the sample surface, which allows for correlation of MS and optical images. The orthogonal geometry of the ion source provides for good separation of direct ion signal and postionized signal by varying the extraction delay, leading to enhanced mass resolution. Use of LDPI-MS is demonstrated for the detection of antibiotics within intact bacterial biofilms.[2] Staphylococcus epidermidis is a common Gram positive bacterium that resides on human skin and is one of the most frequent culprits behind hospital acquired biofilm infections. Treatment of biofilm infections is hindered by the limited ability of antibiotics to kill biofilm associated microbes. LDPI-MS is used to detect antibiotics within intact biofilms without significant interference from other biofilm chemical constituents. Sulfadiazine is detected these biofilms at relatively high concentrations while tetracylcine is detected at near clinical concentrations. These results indicate that imaging MS of bacterial biofilms, animal tissue, or other biological samples by LDPI-MS can be applied to analysis of low ionization potential analytes such as the described antibiotics and other compounds including pharmaceuticals.

[1]M. Zhou, C. Wu, A. Akhmetov, P.D. Edirisinghe, J.L. Drummond and L. Hanley, J. Amer. Soc. Mass Spectrom. 18, 1097 (2007).

[2]G.L. Gasper, R. Carlson, A. Akhmetov, J.F. Moore and L. Hanley, Proteom. (2008) in press.

4:00pm **BO+AS+BI-WeA8 3D Mass Spectrometric Analysis of Non-Dehydrated Biological Samples**, ***H.F. Arlinghaus***, *J. Möller, C. Kriegeskotte, D. Lipinsky*, Westfälische Wilhelms-Universität Münster, Germany

ToF-SIMS and laser-SNMS are increasingly important tools for analyzing the elemental and molecular distribution in biological samples. However, in-vivo analyses of tissues or cell cultures are impossible because the sample must accommodate the vacuum conditions of the instrument. Thus, fixing the sample in its vital state, such as freezing, is essential. Sample preparation by cryo-fractioning or cryosectioning techniques followed by freeze-drying has been successfully used. However, these techniques exhibit several limitations. In order to overcome these limitations, a combination of a ToF-SIMS/laser-SNMS instrument and an in-vacuum cryosectioning instrument were developed for directly preparing and analyzing frozen non-dehydrated samples. The correct sample temperature after preparation and during the analysis showed to be a major factor for the quality of suitable ToF-SIMS/Laser-SNMS analyses. On the one hand, it is necessary to keep the sample cold to avoid freeze drying, on the other hand, a slight increase of the sample temperature removes adsorbates formed by residual gas in the cutting chamber and, more importantly, water resulting from the cutting process, and anticipates adsorption during the analysis. In our presentation, we will show and discuss how to optimize the sample's temperature, sample preparation techniques for analyzing various biological samples, and the possibility of obtaining 3D molecular images of frozen non-dehydrated biological samples. Our data will show that both TOF-

SIMS and Laser-SNMS are capable of imaging elements and molecules in complex biological samples and that they are very valuable tools in advancing applications in life sciences.

4:20pm **BO+AS+BI-WeA9 Construction of Complex Two- and Three-Dimensional Nanostructures: Combined Time-of-Flight Secondary Ion Mass Spectrometry and Microscopy Studies**, *C. Zhou, P. Lu,* ***A.V. Walker***, Washington University in St. Louis

Methods for the chemically selective deposition of metals, semiconductors, biomolecules and other compounds have been studied and applied to the construction of complex multilayer structures. This work has important applications in molecular and organic electronics, sensing, biotechnology and photonics. To illustrate our approach we present two examples: the chemical bath deposition (CBD) of zinc sulfide on functionalized SAMs and the construction of three-dimensional nanostructures via layer-by-layer growth. CBD is a solution-based method for the controlled deposition of semiconductors. ZnS is a direct band-gap semiconductor used electroluminescent devices and solar cells. Two sizes of crystallites are observed to form: ~500 nm nanoflowers and ~2 μm crystallites. Nanoflowers nucleate at Zn(II)-carboxylate terminal group complexes on -COOH terminated SAMs. They grow via an ion-by-ion reaction pathway and remain chemically bound to the SAM. In contrast, the micron-sized crystallites form in solution (cluster-by-cluster growth) and are observed on all SAMs studied (-OH, -COOH and $-CH_3$ terminated SAMs). These crystallites can be easily removed from the surface using sonication. Thus under the appropriate experimental conditions ZnS can be selectively deposited onto -COOH terminated SAMs. We illustrate this by selectively depositing ZnS on a patterned -COOH/$-CH_3$ terminated SAM surface. Our approach for the construction of three-dimensional nanostructures begins with a single SAM layer deposited and UV-photopatterned using standard techniques. A second layer is then assembled by specific chemical reaction with the terminal groups of the first SAM. Additional layers are deposited using the same method. Experiments to date have focused on the specific coupling reaction of amines with carboxylic acids to form multilayer structures. Using TOF SIMS imaging we have followed each step of this reaction and have been able to demonstrate the selective formation of multilayer structures on patterned -COOH/$-CH_3$ surfaces. This coupling is used to make several proof-of-concept multifunctional structures.

4:40pm **BO+AS+BI-WeA10 TOF-SIMS Analysis of Lipid Transfer between Vesicles and Supported Lipid Bilayers on $TiO_2$**, ***P. Sjovall***, SP Technical Research Institute of Sweden, *A. Kunze, B. Kasemo, S. Svedhem*, Chalmers University of Technology, Sweden

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) was used to investigate the electrostatically driven lipid transfer between negatively charged vesicles (POPS) and a positively charged supported lipid bilayer (POEPC) on a $TiO_2$ surface. Quartz crystal microbalance with dissipation (QCM-D) was used to monitor the formation of the POEPC bilayer (by vesicle adsorption and rupture), the subsequent interaction with POPS vesicles, and the resulting lipid transfer between the bilayer and the vesicles. In addition, QCM-D showed that SDS treatment of the bilayer after lipid transfer removed mass corresponding to one of the lipid leaflets (seemingly leaving a lipid monolayer), and that a bilayer could be reformed upon POEPC vesicle adsorption on this monolayer. TOF-SIMS analysis using $Bi_3^+$ primary ions was used to provide quantitative estimates of the lipid composition in the different lipid layers. The lipid bilayers were prepared for TOF-SIMS analysis by plunge freezing and freeze drying(1). In order to allow for unambiguous detection of POPS in the lipid bilayers, POPS with fully deuterated palmitate fatty acid tail groups was used in the buffer vesicles. Quantitative estimates of the lipid compositions were made based on the signal intensities from the deuterated (POPS) and undeuterated (POEPC) palmitate ions, as well as from the oleate (POPS and POEPC) ion, using a simple model for the concentration-dependent TOF-SIMS signal intensities. Reference bilayers prepared with known lipid compositions were analysed in order to provide calibration points for the quantitative analysis. The results show that the POEPC bilayer after lipid transfer contains approximately 50% POPS, while the SDS-resistant monolayer contains about 70% POPS and the reformed bilayer contains 20-25% POPS. Possible asymmetries in the lipid composition of the bilayers were, however, not taken into account. A number of peaks, which can be assigned to molecular ions of POPS, were observed in the negative ion spectra of the POPS-containing lipid bilayers. Interestingly, these peaks were absent in the spectra from the SDS-resistant monolayer, although the signal from the deuterated palmitate ion indicated about 70% POPS in the monolayer. This suggests that the POPS molecular peak only shows appreciable intensity in TOF-SIMS spectra from the bilayer structure, similar to what has been observed previously for POPC.[1]

[1]Prinz et al., Langmuir 2007, 23, 8035-8041.

5:00pm **BO+AS+BI-WeA11 Spatial Distribution Analysis of a Selenium Based Anti-Cancer Drug in Tumor Tissue Samples by ToF-SIMS**, ***S.A. Burns***, University at Buffalo, *M. Khin, L. Kazim, Y. Rustum, S. Cao, F. Durrani,* Roswell Park Cancer Institute, *J.A. Gardella*, University at Buffalo

Time of Flight Secondary Ion Mass Spectrometry (ToF-SIMS) has recently found new applications in the field of tissue analysis due to the advancement of cluster ion sources.[1] Bi cluster primary ion sources have been shown to produce chemical images with high spatial resolution, to ca. 100nm. The use of C60 cluster ion sources for depth profile analysis distributes the ion impact force allowing for a more specialized analysis of organic samples.[2] ToF-SIMS is an extremely useful application to detect low molecular weight drugs within a polymeric membrane. This study utilized this application to spatially image drug distribution of an anticancer agent in a two types of tissue samples. Nude mice implanted with human head and neck tumors were treated with methylselenocystein (MSC), a known anticarcinogen.[3] MALDI imaging has been used to determine the distribution of another anti-cancer drug, CPT-11 when MSC has also been introduced.[4] The distribution of CPT-11 was found to be more even throughout the tumor in the presence of MSC. This indicates that the MSC causes an increase the vasculature of a tumor thereby allowing other anti-cancer drugs to distribute evenly. MALDI imaging was able to show drug distribution but could not associate the fragment peak of the MSC with the vasculature of the cells due to restrictions in image resolution (100μm). ToF-SIMS imaging has been shown to reach resolutions of 100nm allowing for this type of analysis to be performed. MSC treated tumor and liver samples were analyzed using imaging and depth profiling to determine the distribution of drug with respect to the vasculature of the tissue. The first step of this study was to determine characteristic peaks from the MSC that could be identified in the tissue samples. Analysis of the livers and tumors of the mice that had been treated with MSC had fragment peaks with isotopic distributions indicating selenium containing organic compounds not found in the control samples. These fragment peaks were used as the drug peaks whose distribution in the tissue samples were compared to fragment peaks which could be attributed directly to cell vascular structure.

[1]Brunelle,A,et al. Journal of Mass Spectrometry 2005,40,985-999
[2]Fletcher,JS,et al. Analytical Chemistry 2006,78,1827-31
[3]Azrak,RG,et al. Biochemical Pharmacology 2007,73,1280-1287
[4]Prieto Conaway,MC,et al. Thermo Scientific 2008,Application Note.

5:20pm **BO+AS+BI-WeA12 Effects of Different Sample Preparation Methods for Cell Imaging using TOF-SIMS**, ***J. Malm***, SP Technical Research Institute of Sweden, *D. Giannaras*, University of Glasgow, UK, *P. Sjövall,* SP Technical Research Institute of Sweden, *N. Gadegaard, M.O. Riehle*, University of Glasgow, UK

Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is increasingly being used for chemical imaging of cells and tissue. A concern in these studies is that the samples need to be prepared for the vacuum environment. Several sample preparation methods exist for this purpose. In this work, effects of different preparation methods on the structure and surface chemistry of human fibroblast hTert cells were studied. Two fixation protocols, using glutaraldehyde (GA, $C_5H_8O_2$), and osmium tetroxide ($OsO_4$), respectively, were compared to a non-fixing protocol where cells were washed with ammonium formate (AF, $NH_4HCOO$) prior to drying. Three drying techniques were compared, namely freeze-drying (FD) after rapid plunge-freezing, critical point-drying (CPD), and alcohol ladder-drying (ALD). Imaging TOF-SIMS with $Bi_3$ cluster primary ions was used to compare the different preparation protocols with respect to surface chemistry, and the structure of the cells after preparation was studied using scanning electron microscopy (SEM). For the AF-washed samples, changes in cell volume was followed by interference reflection microscopy (IRM). The results show that both the fixation/washing protocols as well as the drying protocols affect the chemical information obtained in TOF-SIMS analyses. For GA-fixed samples, both CPD and ALD give rise to reduced phosphocholine (PC) signal on the cell surface by two orders of magnitude, as compared to FD, while no significant differences are seen for cholesterol and amino acid fragment ions. GA-fixed samples post-fixed using $OsO_4$ showed PC intensities reduced by only one order of magnitude, going from FD to CPD or ALD. The cholesterol intensity was found to be higher for AF-washed cells and cells fixed with $OsO_4$, than for GA fixed cells. An increase in amino acid intensity going from AF to GA to $OsO_4$ was also observed.

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