

Biomaterial Interfaces Division Room 12 - Session BI+AS-WeA

In Honor of Dave Castner's 65th Birthday: Multitechnique Bio-Surface Characterization II

Moderators: Lara Gamble, University of Washington, Daniel Graham, University of Washington

2:20pm **BI+AS-WeA-1 Contributions Advancing Surface Technologies: NEXAFS, ESCA, Rhodium (and More), Buddy D. Ratner**, University of Washington, Seattle **INVITED**

The broad impact that surface science has had on so many technologies is mirrored by the contributions of Professor David Castner to many subfields dependent upon surface science. Dave's earliest contributions to the scientific literature were associated with the surface science of rhodium, iron and cobalt catalysts. Papers were published addressing CO hydrogenation, Fischer-Tropsch polymerization and related topics with relevance to energy consumption and chemical production. With Dave's arrival at the University of Washington in 1986, the subjects of his research shifted from catalysis to biomedical surfaces. Dave and I have always shared a common interest (maybe passion). That is, generating quality data and extracting maximum information from that data. We both had extensive experience with early HP5950 electron spectroscopy for chemical analysis (ESCA) instruments. These monochromatized instruments generated exceptionally high resolution spectra for that era, and the instruments had effective charge compensation for insulators. This allowed us to make great strides in highlighting the use of ESCA for bio-relevant surfaces and biological materials. The theme of data quality has persisted into the present with newer ESCA instruments and then SIMS instrumentation. Dave Castner has taken surface analysis into the 21st century with studies on cells, proteins, novel polymer surfaces and nanomaterials. This talk will highlight Dave Castner's remarkable contributions to surface science with particular emphasis on his contributions to the evolution of methods available to analyze complex surfaces and morphologies.

3:00pm **BI+AS-WeA-3 Characterization of Bio-Molecules with GCIB-SIMS equipped with MS/MS Spectrometer, Jiro Matsuo, T Seki, T Aoki**, Kyoto University, SENTA, JST, Japan

Secondary ion mass spectrometry (SIMS) is now widely used for chemical analysis of polymers and biological materials that have a rather complicated molecular structure. Various types of primary ion beams and mass spectrometers have been developed and used in an attempt to improve sensitivity, as well as lateral and mass resolution. Large gas cluster ion beams (GCIB) have been commercialized for surface analysis techniques, such as SIMS and XPS. Molecular depth profiling and three-dimensional analysis have been applied on organic devices and biological materials. A large cluster ion beam could overcome the limitation of ion dose, which is the biggest obstacle for obtaining more signals in static SIMS.

To expand the applications of the SIMS technique, we have developed a finely focused large cluster ion beam (~1mm) for the primary ion beam for use in SIMS [1] and combined it with mass spectrometers of the quadrupole time-of-flight mass spectrometry (Q-TOF) type without pulsing primary ions. This mass spectrometer is equipped with MS/MS capability and allows to determine the structure of the secondary ion by using the collision-induced dissociation (CID) technique. This is a new SIMS instrument that helps in the characterization of biomolecules in cells, tissue and medicine. For instance, the detection limit of a drug molecule is improved by using the MS/MS technique, because of a much-reduced background.

In this paper, we demonstrate the capability of SIMS with the MS/MS spectrometer to determine the structure of molecular-related ions and discuss the benefits and drawbacks of this technique.

[1] J. Matsuo, S. Torii, K. Yamauchi, K. Wakamoto, M. Kusakari, S. Nakagawa, M. Fujii, T. Aoki, and T. Seki, *Appl. Phys. Express*, **7** (2014), 056602

3:20pm **BI+AS-WeA-4 Linking Nanosilver (AgNP) Toxicity to the Physicochemical Properties of the Particles which can Change as a Function of Experimental and Biological Conditions, Donald Baer**, Pacific Northwest National Laboratory; *J Brown*, University of Colorado at Denver; *A Porter*, Imperial College London, UK; *B Thrall*, Pacific Northwest National Laboratory; *T Tetley*, Imperial College London, UK, United Kingdom of Great Britain and Northern Ireland; *L Van Winkle*, University of California at Davis; *T Xia*, University of California at Los Angeles

Although colloidal Ag is generally considered safe for humans, use of nanosilver in consumer products has dramatically increased both the amount of Ag exposure and possible exposure pathways. To fill knowledge gaps for nano-Ag safety assessment, the National Institute of Environmental Health Sciences supported a consortium of investigators to examine how physical and chemical characteristics of AgNPs can lead to adverse health outcomes. Here we report a consortium perspective linking physicochemical properties of the particles to Ag biodistribution and toxicity. It is necessary to recognize the dynamic nature of AgNPs. They can change in response to handling and variations in their environment and such changes can influence Ag biodistribution and biological responses. Consortium studies identified the critical relationships among AgNP properties, environmental effects, and the biodistribution and fate of Ag associated with the particles. Three critical regions of interactions were identified: i) effect of exposure medium and biological environment on particle properties and transformations; ii) processes occurring at the cellular surface impacting particle attachment, uptake, accumulation and clearance; and iii) particle fate and transformations within a cell. The nature of AgNPs during biological exposure is influenced by the initial characteristics of the particles including size, structure and the presence of designed or inadvertent coatings. These initial properties are usually altered by exposure to artificial or natural media. These physicochemical properties are often time dependent and such changes, including often ignored effects due to handling or storage, can influence biological outcomes. Ag can be transported into cells as both ions and particles. While ions are known to impact cytotoxicity, AgNPs within cells often have greater toxicity. Intercellular processes are similar to those in extracellular media except that the Ag is located within specific microenvironments within a cell. It appears that intracellular dissolution of Ag is a major cause of toxicity.

4:20pm **BI+AS-WeA-7 Protein Imaging from the Subcellular Level to the Single Protein Level, DaeWon Moon**, DGIST, Republic of Korea

Most of biological story tellings are mainly based on proteins and their interactions. Therefore protein imaging and their interaction studies have been the key interest in bio imaging. Most of protein bioimaging have been based on confocal fluorescence microscopy for 2 or 3 proteins. We have developed a new multiplex protein imaging method for TOF-SIMS with metal oxide nanoparticle (MONP) conjugated with proteins up to 9 proteins, in theory, several tens, and a single protein imaging technique based on He Ion Microscopy (HIM)

In SIMS analysis, MONPs provide high secondary ionization yield and amplification of ion yields. We synthesized 9 MONPs working right such as CoO, CdO, Fe₃O₄, TiO₂, PbO, In₂O₃, SiO₂, Al₂O₃, La₂O₃. In addition to protein imaging, SIMS intrinsically provides tens of bio-molecular imaging including lipids and metabolites, and metals with a TOF mass analyzer, which makes this new methodology to be an omni-molecular mass spectrometric imaging technique. Sliced and cultured mouse hippocampal tissues were imaged with typical spatial resolution of 2 μm, which can be improved down to 300 nm for 9 neuronal proteins. Proteins chosen to image mouse hippocampal tissues are NeuN for all nuclei, Cav1.3 for neuron cells, Iba1 for microglia cells, GFAP for astrocytes, AMPA receptor, phosphorylated Tau, amyloid beta (Aβ) 1-42, amyloid precursor protein, and APOE, which were selected to visualize important proteins as landmarks of Alzheimer Disease (AD). With multiplex proteins imaging, we could estimate the proximity of associated proteins in mouse hippocampal tissues, which changes with aging and AD progression.

Since HIM has a spatial resolution of 0.5 nm, HIM can observe single proteins in theory but in practice, it may be very difficult to observe a single protein molecule due to the similar secondary electron yields of proteins compared to other proteins or extracellular matrix molecules. We demonstrated that HIM can image each MONP conjugated with proteins from a mouse hippocampal tissue revealing the distribution of single proteins in synapses, neuronal soma, amyloid plaques, and neurofibrillary tangles with their changes along aging and AD.

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With the co-development of multiplex protein SIMS imaging and single protein HIM imaging technology, I expect we can improve our understanding on the role of proteins and their interactions in biology, biomaterials, and medicine.

4:40pm **BI+AS-WeA-8 Integrating Biological and Surface Chemical Characterisation to Probe Bacterial and Lipid Vesicle Interactions at Surfaces**, *Sally McArthur*, Swinburne University of Technology and CSIRO, Australia; *M Abrigo, H Askew, K Jarvis*, Swinburne University of Technology, Australia

Control and the ability to elicit specific responses from a biological system lies at the heart of most bioengineering. We want to immobilize proteins on biosensors but ask them to behave as they would in the body, stimulate cells to assemble tissues, form new blood vessels and replicate structures in the lab just as well as they can in our bodies. We want methods that prevent bacteria forming biofilms and better still we would like them to stop attaching to surfaces full stop. We have an armada of techniques at our disposal, surface engineering, macro, micro and nanomaterials, drugs and biomolecules, light, electricity and a plethora of analysis tools to give us new insight into how the systems we build behave. But as we increase the complexity of the system, we need to be able to match this with combinations of characterisation techniques that probe both the biological and physicochemical processes occurring at the biointerface.

This talk will explore how we utilise QCM, XPS, ToF-SIMS, fluorescence imaging and biological assays to investigate the influence of surface chemistry and micro and nanoscale topography on interactions with lipid vesicles and bacteria.

5:00pm **BI+AS-WeA-9 A Physical Chemist and a Chemical Engineer Walk into a Bar... Reflections on Surface and Interface Analysis**, *Matthew Wagner*, The Procter & Gamble Company **INVITED**

Surface and interface science is critical to many applications across many industries, spanning from advanced technologies in microelectronics and biomaterials to everyday household goods such as laundry detergents and shampoos. Micro and nanoscale phenomena at surfaces and interfaces, including adsorption, wetting, self-assembly, and many others, drive macroscale performance, resulting in significant benefits when done well and significant failures when poorly understood or controlled. At all scales, measurement science specific to surfaces and interfaces is critical to understanding these phenomena.

In the field of biomaterials science (and beyond), protein adsorption is a foundational step in all interactions between biological systems and synthetic materials. Many surface analysis techniques have been applied to the characterization of adsorbed protein films, including understanding the amount, composition, spatial distribution, and orientation of adsorbed proteins. In this special session in honor of Dave Castner, this presentation will review key contributions from the Castner group on the application of multi-technique surface analysis techniques to adsorbed protein films. In particular, the use of ToF-SIMS and multivariate data analysis techniques in conjunction with complementary surface spectroscopies including XPS, NEXAFS, SPR, and others, will be reviewed. The broader impact of these developments in surface analysis methodologies on the fields of surface and interface science across industries will be discussed.

5:40pm **BI+AS-WeA-11 Investigating the Cytotoxicity of Commercially Available Poly(*N*-isopropyl Acrylamide)-coated Surfaces**, *L Stapleton, M Cooperstein, P Nguyen, Heather Canavan*, University of New Mexico

Poly (*N*-isopropyl acrylamide) (pNIPAM) is a thermoresponsive polymer that undergoes a phase change at a physiologically relevant temperature range, which leads to mammalian cell release. Below its lower critical solution temperature (LCST $\sim 32^\circ\text{C}$), pNIPAM becomes hydrated and is hydrophilic. In this state, its chains become extended and cells detach as intact cell sheets. Before the detached cell sheets can be used on humans, the cytotoxicity of the surfaces must be assessed. In previous studies, we found that although most techniques for polymerizing NIPAM (e.g., plasma polymerization, ppNIPAM; and sol-gel preparations of NIPAM, spNIPAM) yielded biocompatible films, those from commercially available NIPAM (cpNIPAM) were relatively cytotoxic. In this work, we investigate the reasons behind this anomaly. The cpNIPAM-coated surfaces were evaluated for their thermoresponse and surface chemistry using standard surface science techniques (e.g., goniometry, X-ray photoelectron spectroscopy). The relative biocompatibility of the substrates with cultured bovine aortic endothelial cells (BAECs) and monkey kidney epithelial cells exposed to extracts from the cpNIPAM, spNIPAM, and ppNIPAM films was assessed using pop off experiments and Live/Dead assays. In addition, the

extract solutions themselves were analyzed by NMR and mass spectroscopy. We find that the diminished cell viability of BAECs exposed to cpNIPAM substrates is due to a combination of factors, including the inclusion of short chain length polymers and the presence of unreacted catalyst. This work will have valuable insights into the cytotoxicity of cpNIPAM-coated surfaces, and therefore, into the applicability of cells grown on this surface for human subjects.

6:00pm **BI+AS-WeA-12 Development of Surface Analysis Methods for Characterizing Immobilized Proteins**, *David Castner*, University of Washington

One of the first events that occurs when a biomedical device is placed in the biological environment is the interactions of proteins with the surface region of the biomedical device. How the proteins interact with the surface can have a significant impact on further biological responses in both *in vivo* and *in vitro* applications. Thus, it is essential to understand how proteins interact with surfaces and any structural modifications they undergo as a result of these interactions. Key objectives for characterizing surface-bound proteins are (1) identifying the type of proteins bound to the surface, (2) determining the amount of each surface-bound protein, (3) determining the conformation and orientation of the bound proteins and (4) characterizing the spatial distributions of surface-bound proteins. There are many bonding mechanisms for attaching proteins to surfaces (charge-charge, coordination complexes, covalent bond formation, ligand interactions, etc.). Each method has its advantages and disadvantages. How the protein structure, especially its conformation and orientation, is affected by surface attachment will be a function of the surface structure and composition of the biomaterial as well as properties of the protein. There are often time-dependent changes in the composition, conformation, orientation, and distribution of the complex, multi-component protein films deposited from the biological environment. So the structural determinations for surface bound proteins need to be related not only to the properties of the biomaterial surface and protein, but also to the experimental conditions used to attach the protein to the surface. Results using experimental methods (XPS, ToF-SIMS, SFG, SPR, QCM-D, etc.) combined with computation methods (e.g., MD simulations) provide important information about the attachment, specificity, orientation, conformation and spatial distribution of surface immobilized proteins. This talk will discuss the significant progress has been made in developing surface analysis methods for characterizing the structure of surface immobilized proteins as well as the current challenges. Future protein characterization studies need to be extended to more complex samples as well as more tightly integrating complementary techniques that can be used to directly study immobilized proteins in the presence of the biological environment. In addition, further advances in computational methods for predicting protein-surface interactions and structures as well as providing structural information at the atomic level for large biomolecules is needed.

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