

Biomaterial Interfaces

Room 209 F W - Session B11-MoM

Characterization of Biological and Biomaterials Surfaces

Moderators: Pierluigi Bilotto, TU Wien, Morgan Hawker, California State University, Fresno

8:15am B11-MoM-1 Determine Protein Conformation and Orientation at Buried Solid/Liquid Interfaces in Situ, *Zhan Chen*, University of Michigan

INVITED

Interfacial protein properties play important roles in many research areas and practical applications, such as biomedical materials, marine antifouling coatings, membranes for biological molecule separation, biosensors using surface immobilized enzymes, and antibody drug manufacturing and storage, etc. The properties of proteins at interfaces are determined by molecular structures of interfacial protein molecules. In this study, a nonlinear optical laser spectroscopic technique, sum frequency generation (SFG) vibrational spectroscopy, has been used to determine conformations and orientations of proteins at buried solid/liquid interfaces in situ in real time. A combined approach using molecular dynamics simulation, SFG experimental data, Hamiltonian spectra calculation, spectra matching, and isotope labeling was used for interfacial protein structure determination in this research. This method was successfully applied to study protein Gb1 adsorption to a variety of substrates, interfacial antibody – surfactant interactions, protein dimer formation at interface, membrane protein complex structure, and time-dependent protein structural change during the adsorption process.

8:45am B11-MoM-3 Cryo-XPS Characterisation and Solution Realism for Functional Nanoparticle Analysis, *Liam Soomary, Jonathan Counsell*, Kratos Analytical Limited, UK; *David Cant, William Lee*, National Physical Laboratory, UK

A crucial part of nanoparticle engineering relies on understanding and controlling surface functionalisation. Traditionally, analysis can be performed with techniques such as Transmission Electron Cryomicroscopy (CryoTEM) [1], however quantitative surface characterisation remains a challenging prospect.

X-ray Photoelectron Spectroscopy (XPS) has long been an exemplary technique for quantitative surface analysis, offering high sensitivity to elemental compositions and chemical states. However, its requirement for ultra-high vacuum (UHV) often compromises the relevant conditions under which most organic nanoparticle systems operate, leading to questions about their morphology and stability of their functionalised groups once the solvent environment is removed [2]. Recent developments in cryogenic XPS (Cryo-XPS) aims to bridge this gap. Through flash-freezing, liquid nanoparticles can be preserved in a close-to-native state within UHV conditions, minimising environment induced changes and enabling insights without significant structural perturbations [3].

In this talk, we discuss complementary techniques for solution-based measurements and highlight the benefits of Cryo-XPS in probing functionalised nanoparticles. Special attention is given to PEG-coated nanoparticles, which are widely used in drug delivery systems and biomaterials research. As we illustrate – through a case study of lipid nanoparticles – how sample preparation, handling and methodology can improve quantitative surface analysis of these systems.

[1] Judith Kuntsche *et al.*, *Cryogenic transmission electron microscopy (cryo-TEM) for studying the morphology of colloidal drug delivery systems*, International Journal of Pharmaceutics, (2011), 120-137, DOI: 10.1016/j.ijpharm.2011.02.001

[2] S. Mourdikoudis *et al.*, *Characterization techniques for nanoparticles: comparison and complementarity upon studying nanoparticle properties*, The Royal Society of Chemistry, (2018), 12871-12934, DOI: 10.1039/C8NR02278J

[3] G. Weisenberger *et al.*, *Understanding the invisible hands of sample preparation for cryo-EM*, Nat. Methods, (2021) 18:5, DOI: 10.1038/s41592-021-01130-6

9:00am B11-MoM-4 GCIB-SIMS in the study of Lymphoma, *John Fletcher, Simon Uzoni, Noora Neittaanmäki, Vasilis Chatzikyriako, Daniele Zanchin*, University of Gothenburg, Sweden

The advent of gas cluster ion beams (GCIBs) for SIMS has greatly benefited the analysis of biological samples through the generation of increased

intact molecular secondary ions. This has enabled detailed molecular maps to be generated in order to perform "molecular pathology", elucidating chemical changes associated with different diseases. In this study GCIB-SIMS, in this case using a 40 keV $(\text{CO}_2)_n^+$ ion beam on a J105 ToF-SIMS instrument (Ionoptika Ltd.) was used to map the intact lipid signals across 14 human lymph node samples representing diffuse large B-cell lymphoma (DLBCL) and control samples. DLBCL is a common and aggressive form of lymphoma resulting in a diffuse distribution of cancerous cells amongst the typical lymph cells. The analysis allowed the samples to be classified as malignant or non-malignant and also highlighted additional aggressive cancer signature in a DLBCL sample with an unusually high proliferation index. A complementary, combined k-means/image PCA approach was used to interrogate the data highlighting the pros and cons of the different approaches and potential sources for misclassification/diagnoses resulting from the heterogeneity of the DLBCL samples. Compared to other cancer samples the lipid markers associated with cancer can appear reversed as many studies have classed inflammatory responses to cancer as part of the cancer signature. In the lymph node tissue, the onset of malignant transformation is associated with a decrease in inflammatory character. While delivering new information regarding the chemistry of lymphoma the results also highlight the need for cellular precision with high chemical specificity and sensitivity, and the challenges associated with spectral/spatial classification of such complex samples and data where differently aggressive cancer samples show different signatures and pockets of different cell types, in this case histiocytes, can be show intermediate cancer/healthy lipid profiles.

9:15am B11-MoM-5 Optical Dynamics of Electrochemically Driven Reflectin Protein Films, *Yin-Chen Lin, Dan Morse, Lior Sepunaru, Michael Gordon*¹, University of California at Santa Barbara

Near- and sub-wavelength photonic structures are used by different organisms (e.g., insects, cephalopods, fish, birds) to create vivid and often dynamically-tunable colors, as well as create, manipulate, or capture light for vision, communication, crypsis, photosynthesis, and defense. This talk will highlight our work to understand and translate the biological mechanism of reflectin, an intrinsically disordered protein found in squid skin cells that is responsible for dynamically tunable structural color, into new materials and device venues with the ultimate goal of using biological components and paradigms to create novel multi-scale structures with functional properties. Neuronally triggered-phosphorylation drives the condensation of reflectin proteins *in vivo*, resulting in osmotic dehydration of cell membrane-encapsulated layers of reflectin-loaded lamellae and low refractive index extracellular space that effectively function as a biological and tunable distributed Bragg reflector (DBR).

In close analogy to this physiological phenomenon, we demonstrate here that electrochemical reduction enables tunable and reversible control of reflectin condensation and thin film water fraction, allowing one to electrochemically tune reflectin film refractive index and thickness, just as that occurring in the squid [1]. Electrochemical correlative ellipsometry and surface plasmon resonance spectroscopy were developed to trigger and simultaneously analyze the dynamic changes in optical properties of reflectin films to further elucidate and mimic the color-changing mechanisms in squid skin. Measurements indicate that electrochemical reduction allows precise modulation of film refractive index (1.36 to 1.40) and thickness (40-100 nm). Condensation-driven, cyclical FRET emission from reflectin films is also demonstrated using electrochemical triggering as a preface to implementing reflectin as a triggerable optical medium in 1D gratings. Overall, this work opens new approaches to analyze biophysical mechanisms governing protein condensation and structural color regulation, and facilitates the design of bio-enabled functional materials and devices that bridge the biotic-abiotic gap.

[1] Y.-C. Lin, C. Yang, S. Tochikura, J.R. Uzarski, D.E. Morse, L. Sepunaru, and M.J. Gordon, *Advanced Materials* 2411005 (2025).

9:30am B11-MoM-6 Interaction Dynamics of Borrelia Surface Proteins with Fibronectin, *Nuri Oncel, Kavindi Hewage, Carlos Munoz, Mehmet Ozdogan, Catherine Brissette*, University of North Dakota

Lyme disease, caused by the bacterium *Borrelia burgdorferi*, is a significant public health concern in North America, with approximately 500,000 cases reported annually in the United States. The dissemination of *B. burgdorferi* from the initial tick bite site to various tissues is facilitated by surface adhesins that bind to extracellular matrix (ECM) proteins such as

¹ JVST Highlighted Talk

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fibronectin (Fn). This study investigates the binding dynamics of *B. burgdorferi* surface proteins RevA, BBK32, BmpA, OspA, and OspC to Fn using atomic force microscopy-based single-molecule force spectroscopy (AFM-SMFS). Our results demonstrate that RevA and BBK32 form strong, stable bonds with Fn, highlighting their roles as key mediators of host-cell attachment. By quantifying the rupture forces and kinetic parameters of these interactions, we provide a deeper understanding of *B. burgdorferi* adhesion mechanics and offer insights into potential therapeutic strategies targeting early bacterial attachment.

9:45am **BI1-MoM-7 Ready, Settlement, Action! Leveraging Microcomputed Tomography in Barnacle-Substrate Interactions, *Brittney Mitchell*, *Beatriz Orihuela*, Duke University; *Gary Dickinson*, The College of New Jersey; *Daniel Rittschof*, Duke University**

Barnacle fouling is a persistent challenge in marine environments, contributing to loss of performance, increased fuel costs, material degradation and substantial economic costs. Human and environmental health concerns are driving regulations and interest in transitioning away from broad biocidal antifouling towards surface-engineered materials that resist biofouling through physical and chemical modifications. Optimizing these materials requires robust, quantitative and qualitative insight into interactions between barnacles and substrates. The barnacle-material interface is dynamic whether looking down from the barnacle or up from the surface. Barnacles attack surfaces with adhesives, enzymes, metabolites, surfactants and reactive oxygen species. Surfaces leach chemicals, contaminants, catalysts and additives, many of which are biologically active orders of magnitude below engineering additions. We apply high-resolution microcomputed tomography (μ CT) to visualize and quantify intact barnacle morphologies and surface architecture in 3D macro- to microscale domains. We provide detailed evaluation of exoskeletal and adhesive plaque morphology, as well as substrate conformity or modification. Reconstructions of scans into visual media helps facilitate comparative analysis across substrate types and species. Our data show substrate-driven plasticity in barnacle plate and adhesive plaque phenotypes and evidence of substrate alteration beneath living barnacles. These findings demonstrate the utility of μ CT and 3D visualization as tools for comprehensive characterization of biofouling interactions.

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