Biomaterial Interfaces
Room 117 - Session BI2-TuM

Characterization of Biological and Biomaterial Surfaces II: Celebration of Stephanie Allen

Moderators: Morgan Hawker, California State University, Fresno, **Sapun Parekh**, University of Texas at Austin

11:00am **BI2-TuM-13 Biointerfacial Characterisation of Implanted Medical Devices with OrbiSIMS***, **Morgan Alexander**,* University of Nottingham, UK

The 3DOrbiSIMS hyphenation of ToF SIMS with an OrbiTrap™ makes meaningful analysis the molecules in complex biological samples and bio-interfaces formed on materials feasible. [1,2] Critically, the mass resolving power and mass accuracy has rendered routine peak assignment with deviations below 2 ppm. The large spectral data files with thousands of peaks that arise from biological samples requires automated untargeted analysis to make the most of this information. These have been enabled by the methodology for molecular formula prediction (MFP) assignment adapted to SIMS by Edney et al. [3]

I will illustrate how this enables us to investigate the bio-interface for implanted medical devices, to shed light on their failure mechanisms. The importance of the lipids and other metabolites is revealed in the analysis of tissue sections. Subsequent analysis of the bio interfacial deposit at the surface of extracted devices sheds light on the complexity of this process. [4,5] Understanding medical implant fibrosis by biointerfacial OrbiSIMS analysis [Bin Sabri unpublished]

References

1. Mass spectrometry and informatics: distribution of molecules in the PubChem database and general requirements for mass accuracy in surface analysis. FM Green, IS Gilmore, MP Seah (2011) *Analytical Chemistry*.
2. The 3D OrbiSIMS: Label-free metabolic imaging with subcellular lateral resolution and high mass-resolving power. Passarelli et al. (2017) *Nature Methods*.
3. Molecular formula prediction for chemical filtering of 3D OrbiSIMS Datasets. Edney et al (2022) *Anal Chem.*
4. Single-cell metabolic profiling of macrophages using 3D OrbiSIMS: Correlations with phenotype. Suvannapruk et al. (2022) *Anal Chem*
5. Spatially resolved molecular analysis of host response to medical device implantation using the 3D OrbiSIMS highlights a critical role for lipids. Suvannapruk et al. (2024) *Advanced Science*.

11:15am **BI2-TuM-14 SIMS for Label-Free in situ Analysis of Glycosaminoglycans***, **Li Jennifer Lu**,* University of Nottingham, UK*; J. Hippensteel,* University of Colorado - Anschutz Medical Center*; K. Grobe,* University of Münster, Germany*; C. Gorzelanny,* University Medical Center Hamburg - Eppendorf, Germany*; A. Kotowska, D. Scurr, A. Hook,* University of Nottingham, UK

Glycosaminoglycans (GAGs) are linear polysaccharide chains with many varied roles in physiology, including embryonic patterning and modulation of blood vessel permeability. Despite their biological importance, their *in-situ* analysis is limited by a lack of analytical tools with which to study their complex structure. Here we present the development of secondary ion mass spectrometry (SIMS) for *in-situ* GAG analysis [1], allowing for simultaneous spatial and compositional analysis. Initially, a list of characteristic ions for different GAG types was identified using high mass resolution analysis using an Orbi-trap mass analyser of a library of reference GAGs. These GAG-derived ions were validated using a range of biosynthetic enzyme knockout cellular models. This approach has been used to spatially assess the distribution of varied GAG types within complex tissues, including a sepsis model and to explore embryogenesis within *Drosophila*. Additionally, the depth profiling capability of SIMS enables 3D imaging of GAG ions within samples. This demonstrated ToF-SIMS as a powerful analytical tool to spatially analyse (at near optical resolution) GAG type and composition within a single analysis across multiple biological sample types.

References

1.Hook, A.L., Hogwood, J., Gray, E. et al. High sensitivity analysis of nanogram quantities of glycosaminoglycans using ToF-SIMS. Commun Chem 4, 67 (2021).

11:30am **BI2-TuM-15 Force Probe Techniques for Probing Biologic and Lipid Bilayer Interactions Under Physiological Conditions***, **Markus Valtiner**, L. Mears, I. Peters,* TU Wien, Austria

Quantification of biologic interactions - from single molecular to macroscopic interfaces - is essential for understanding function in living systems. We will provide a short overview of force probe techniques (AFM, SFA, and optical tweezers) and will then discuss lipid bilayer interactions, and single molecular interaction measurements (under potential control) in detail. These are essential to a vast range of biological functions, such as intracellular transport mechanisms. Surface charging mediated by concentration dependent ion adsorption and desorption on lipid headgroups alters electric double layers as well as van der Waals and steric hydration forces of interacting bilayer and molecules. Two examples will be discussed:

First, we characterized the interaction between single hydrophobic molecules quantitatively using atomic force microscopy, and demonstrated that single molecular hydrophobic interaction free energies are dominated by the area of the smallest interacting hydrophobe. The interaction free energy amounts to 3–4 kT per hydrophobic unit. Also, we find that the transition state of the hydrophobic interactions is located at 3 Å with respect to the ground state, based on Bell–Evans theory.

Further, we directly measure bilayer interactions during charge modulation in a symmetrically polarized electrochemical three-mirror interferometer surface forces apparatus. We quantify polarization and concentration dependent hydration and electric double layer forces due to cation adsorption/desorption. Results demonstrate that exponential hydration layer interactions effectively describe surface potential dependent surface forces due to cation adsorption at high salt concentrations. Hence, electric double layers of lipid bilayers are exclusively dominated by inner Helmholtz charge regulation under physiological conditions. These results are important for rationalizing bilayer behavior under physiological conditions, where charge and concentration modulation may act as biological triggers for function and signaling.

We will finally provide an outlook on combining all force probe techniques with electrochemical potential modulation.

11:45am **BI2-TuM-16 Tribochemical Nanolithography – Fast, Simple Biomolecular Nanopatterning with 23 nm Resolution at Speeds of up to 1 mm s-1***, O. Siles-Brugge, C. Ma, A. Meijer, **Graham Leggett**,* University of Sheffield, UK

Films formed by the adsorption of (methoxyheptaethylene glycol) nitrophenylethoxycarbonyl-protected aminopropyltriethoxysilane (OEG-NPEOC-APTES) on silica are highly resistant to the adsorption of proteins. On exposure to UV light, the photocleavable protecting group is removed allowing the immobilization of biomolecules.

We have discovered that the same result can be achieved using an AFM probe at a load of ca. 100 nN in the absence of UV light. A FWHM of 23 nm can be achieved at a writing rate of 1 mm s^{-1}. The FWHM increases with load, reaching 90 nm at a load of 10 µN. At larger loads than this an abrupt transition occurs to a regime dominated by mechanical abrasion, yielding broader features. However, for control films that do not contain photo-removable protecting groups, lithographic modification was not observed at loads below 10 µN.

We hypothesize that at low loads the AFM probe causes selective cleavage of the same C-N bond in the carbamate group that is cleaved during UV irradiation. Consistent with this, we found that patterned surfaces can be derivatized with nitrilotriacetic acid (NTA) functional groups, enabling coupling of His-tagged green fluorescent protein (GFP) to the surface. Confocal fluorescence microscopy confirms that GFP attaches to nanolines, but is released when the samples are treated with imidazole, which disrupts the interaction between NTA and the His tag on the protein, consistent with site-specific binding.

The effect of compression on the nitrophenyl protecting group was explored using density functional theory (DFT). Our results indicate that compression of the nitrophenyl group causes substantial changes in its electronic structure. In particular, the energy of the main energetic barrier in the photodeprotection scheme, the initial S_0 to S_1 transition, is greatly reduced, so that deprotection may occur at near IR wavelengths. Hence, application of the AFM probe facilitates deprotection by low energy photons, while UV photons are required in the absence of a mechanical deformation.

The methodology may also be applied to the fabrication of polymer nanostructures. Tribochemical nanolithography of nitrophenylpropyloxyoxycarbonyl protected aminopropyl triethoxysilane (NPPOC-APTES) films yields amine-functionalised nanolines that are functionalized with bromine initiators and used to grow surface-grafted polymer brushes. Polymer chains grafted to the smallest nanolines are collapsed, because they have a high free volume and because adsorption to the surrounding surface is energetically favourable. However, as wider structures are formed, the chains repel each other and begin to swell away from the surface.

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