Tuesday Morning, December 4, 2018

Biomaterial Surfaces & Interfaces
Room Naupaka Salon 6-7 - Session BI-TuM

Bioimaging and Bionanotechnology
Moderator: Lara Gamble, University of Washington

8:00am **BI-TuM1 Exosomes and Extracellular Vesicles: Small Particles with a Big Impact**, ***Renee Goreham,*** Victoria University of Wellington, New Zealand **INVITED**

Extracellular vesicles (EVs), such as exosomes are membrane-bound vesicles released by most living cells and play a vital role in cell function and cell-cell communication. EVs have shown massive potential as biomarkers for a wide range of diseases and are found in most bodily fluids, including blood, saliva, breastmilk and urine. Detection and measuring cell specific EVs in complex solutions can lead to more sensitive detection of diseases, such as cancer. We have synthesised water soluble InP/ZnS (core/shell) quantum dots using optimised ligand exchange methods. Subsequently, the water-dispersible quantum dots were conjugated to EV-specific antibodies or aptamers. The quantum dot-antibody conjugates and their EV binding, were characterised using a suite of techniques to confirm the size, morphology and surface chemistry. The use of non-cadmium-based quantum dots implies that these conjugates would be more viable for use in a clinical setting. The same strategy has also been applied to bacteria cells (i.e. A*cinetobacter baumannii*) and bacteria derived EVs. Combined with custom designed platforms for surface plasmons resonance or spectroscopy detection, we aim to develop novel methods for EV detection.

9:00am **BI-TuM4 The Role of Lipid Surfaces in Molecular Mechanism of Alzheimer's Disease**, *E. Drolle, M. Robinson, B.Y. Lee, C. Filice, S. Turnbull, N. Mei,* ***Zoya Leonenko,*** University of Waterloo, Canada

Alzheimer's disease (AD) is a neurodegenerative disease characterized by dementia and memory loss for which no cure or prevention is available. Amyloid toxicity is a result of the non-specific interaction of toxic amyloid oligomers with the surface of plasma membrane. We studied amyloid aggregation and interaction of amyloid beta (1-42) peptide with lipid model membranes using atomic force microscopy (AFM), Kelvin probe force microscopy (KPFM) and surface Plasmon resonance (SPR). Using AFM-based atomic force spectroscopy (AFS) we measured the binging forces between two single amyloid peptide molecules. Using AFM imaging we showed that amyloid binding and aggregation are affected by charge and polarity of the surfaces (we studied chemically modified inorganic surfaces, phospholipid monolayers and bilayers (membranes)). Furthermore, we demonstrated that lipid membrane surfaces play an active role in amyloid binding and toxicity and thus in molecular mechanism of AD: changes in membrane composition and properties increase amyloid binding to the membrane and membrane damage. Effect of lipid composition, the presence of cholesterol and melatonin are discussed. We discovered that membrane cholesterol creates nanoscale electrostatic domains which induce preferential binding of amyloid peptide, while membrane melatonin reduces amyloid-membrane interactions, protecting the membrane from amyloid attack. These findings contribute to better understanding molecular mechanisms of Alzheimer's disease and aid to the developments of novel strategies for cure and prevention of AD.

References

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2. B.Mehrazma, M.Robinson, S.K.A.Opare, A.Petoyan, J.Lou, F.T.Hane, A.Rauk, Z.Leonenko. Pseudo-peptide amyloid-β blocking inhibitors: molecular dynamics and single molecule force spectroscopy study. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics* 1865 (11), 1707-1718.

3. M.Robinson, B.Y.Lee, Z.Leonenko, Drugs and Drug Delivery Systems Targeting Amyloid-β in Alzheimer's Disease. *AIMS Molecular Science,* 2015, 2(3)*:* 332-358.

4. E.Drolle, R.M.Gaikwad, Z.Leonenko, Nanoscale electrostatic domains in cholesterol-laden lipid membranes create a target for amyloid binding. *Biophysical Journal,* 2012, 103(4), L27-L29.

5. E.Drolle, F.Hane, B.Lee, Z.Leonenko, Atomic force microscopy to study molecular mechanisms of amyloid fibril formation and toxicity in Alzheimer's disease. *J. of Drug Metabolism Reviews*, 2014, 46(2): 207-223.

9:20am **BI-TuM5 An PEEM and Imaging XPS study of Neutrophil Extracellular Traps Caputuring Nanoparticles**, *A. Skallberg, K. Bunnfors, C. Brommesson,* ***Kajsa Uvdal,*** Linköping University, Sweden

Photoelectron Emission Microscopy (PEEM) and Imaging X-ray Photoelectron Spectroscopy (XPS) have the potential to deliver element specific imaging useful for biomedical visualization. This may increase the understanding of biological processes on the cellular level, contributing with element specific information and data on topographical morphology combined. The technique is based on chemical composition, chemical states and work-function shifts.

This is hereby demonstrated by combined PEEM and Imaging XPS investigation of neutrophils and their activation processes. Neutrophils are vital components in the human defense system, with the fundamental role to fight invading pathogens. Neutrophils are also able to release nuclear DNA done by formation of extracellular web-like structures called neutrophil extracellular traps (NETs) to capture and occasionally kill intruding microbes.

Here we reportneutrophils externally triggered by in this case nanoparticle (NPs). The neutrophils and NETs formation are imagined in presence of NPs and we report elemental composition of single-cells and structure of NETs. Active cellular uptake of nanoparticles is imaged both before and after NETs release. Element specific imaging of this novel capability for mass transport. This shows the potential for element specific bio-related cell studies on surfaces and nanoparticle tracking on the cellular level.

9:40am **BI-TuM6 Chemical Imaging of Aggressive Basal Cell Carcinoma using ToF-SIMS**, *M. Munem, K. Dimovska Nilsson,* University of Gothenburg, Göteborg, Sweden*; O. Zaar, N. Neittaanmäki, J. Paoli,* Sahlgrenska University Hospital, Gothenburg*;* ***John Fletcher,*** University of Gothenburg, Göteborg, Sweden

Time-of-fight secondary ion mass spectrometry (ToF-SIMS) is starting to be of increasing value to clinicians and has been used on different tissue samples to successfully identify and localise chemical components to various areas of the tissue and answer disease related questions [1]. Compared to other methods, the main advantage of ToF-SIMS is the label free detection of a large number of different molecules within one experiment on the same tissue section. ToF-SIMS is successfully used for analysing lipids behaviour in biological samples like breast cancer tissue [2]. Basal cell carcinoma (BCC) is one of the most increasing cancers worldwide and it is the most common malignancy in white people. Although the mortality is low as BCC rarely metastasises, this malignancy causes considerable morbidity and places a huge burden on healthcare services worldwide. Furthermore, people who have this condition are at high risk of developing further BCC and other malignancies [3].

Samples were collected from patients with BCC, by Mohs surgery. The tissue was sectioned for ToF-SIMS analysis and H&E staining of consecutive tissue slices was performed. ToF-SIMS was performed using an Ionoptika J105 instrument using a 40 keV $(CO_2)_{6000}^+$ ion beam. The analysis provided detailed chemical information about the individual lipid species and the spatial distribution of these within the tissue. It was possible to observe differences between the layers of the skin as well as between healthy and cancerous tissue (see figure). ToF-SIMS data was correlated with H&E stained images to understand and confirm, from which structures or regions of the tissue that the individual signals originated.

10:20am **BI-TuM8 Combining the Benefits of GCIB-ToF-SIMS, MALDI-FTICR-MS and LC-MS/MS for Location specific Lipid Identification in Planarian Flatworm Tissue Sections**, ***Tina Angerer,*** University of Washington, USA*; D. Velickovic, J.E. Kyle, C. Nicora, C. Anderton,* Pacific Northwest National Laboratory, USA*; D.J. Graham, L.J. Gamble,* University of Washington, USA

Phagocata gracilis are planarian, non-parasitic flatworms. Planarians are best known for their fascinating regenerative abilities, requiring a complex interplay of a wide range of molecules. The regeneration process and the molecules involved are still poorly understood. Most notably there is a lack of lipid and fatty acid data, a diverse group of molecules fulfilling numerous functions such as energy storage and cell signaling.

To gain a better understanding of the lipidomic landscape in planarians we analyzed positive and negative ions from longitudinal sections of *P. gracialis* with MALDI-FTICR-MS and ToF-SIMS along with homogenized whole worm extracts with LC-MS/MS.

Imaging MALDI-FTICR-MS (15T, Bruker Solarix) provides location specific (50 µm/pixel), ultra-high mass resolution (R≈250,000 @m/z=400) and ultra-high accuracy (<1ppm) lipid data capable of distinguishing intact lipid

species of similar exact mass and showing their distribution in the tissue. The drawbacks for this technique are that the spatial resolution is too low to clearly identify features within the worm and its low fragmentation rate. While beneficial for molecular peak intensities, the lack of fragments does not allow for specific lipid assignments with structural information (e.g. lipid headgroup and both fatty acid chains identified).

Imaging GCIB-ToF-SIMS (J105, Ionoptika) generates high mass accuracy (< 5ppm), cell/organ-specific data (3 µm/pixel) consisting of intact lipids, lipid fragments and fatty acids. The moderate mass resolution (R≈10,000@m/z=700) is sufficient to resolve most lipid species. Mass peaks consisting of more than one species are indicated by broad and/or asymmetric peaks with poor mass accuracy. However co-localizing fragments can add confidence for the assignment of overlapping species, provide us with structural information and allow for unambiguous identification of resolved peaks.

LC-MS/MS (Thermo Velos Pro Orbitrap) separates different lipid species prior to fragmentation so, in contrast to SIMS, the observed lipid fragments are guaranteed to stem from the analyzed species. The drawback with this technique is that it provides no location specific information. Similar to SIMS, lipids with similar mass are not separated leading to mixed fragments in the MS/MS data. Comparing LC-MS/MS to SIMS data shows that the same lipid fragment species are present in both spectra.

This work demonstrates that only by correlating all 3 techniques can we get highly accurate, high mass, high spatial resolution, structural and location specific lipid information. Together this data provides detailed information about all major structures and organs within planarians.

10:40am **BI-TuM9 Hybrid SIMS: A New SIMS Instrument for High Resolution Organic Imaging with Highest Mass-resolving Power and MS/MS***, **Nathan Havercroft**,* ION-TOF USA, Inc.*; A. Pirkl,* IONTOF GmbH, Germany*; D. Scurr, N. Starr,* University of Nottingham*; R. Moellers, H. Arlinghaus, E. Niehuis,* IONTOF GmbH, Germany

Introduction

Secondary ion mass spectrometry (SIMS) offers the possibility to acquire chemical information from submicron regions on inorganic and organic samples. This capability has been especially intriguing for researchers with life science applications. In recent years, the vision to image and unambiguously identify molecules on a sub-cellular level has been driving instrumental and application development. While new ion sources expanded the usability of SIMS instruments for biological applications, SIMS analyzers lacked the required mass resolution, mass accuracy and MS/MS capabilities required for the thorough investigation of these materials.

Methods

To specifically address the imaging requirements in the life science field a powerful new Hybrid SIMS instrument [1] was developed in a research project by IONTOF and Thermo Fisher Scientific™, following Prof. Ian S. Gilmore's original idea, in close cooperation with the National Centre of Excellence in Mass Spectrometry Imaging (NiCE-MSI), GlaxoSmithKline, and the School of Pharmacy of the University of Nottingham. The instrument combines an Orbitrap™-based Thermo Scientific™ Q Exactive™ HF mass analyzer with a high-end ToF-SIMS system (IONTOF GmbH). The instrument provides highest mass resolution (> 240,000) and highest mass accuracy (< 1 ppm) with high lateral resolution cluster SIMS imaging.

Preliminary data

Secondary ions, generated by primary ion bombardment from liquid metal ion clusters or large gas clusters can be analyzed in either of the mass analyzers. Fast switching between the mass analyzers is achieved by pulsing of a hemispherical electrode. This even allows combined measurement modes using the TOF for very fast imaging and the Orbitrap mass analyzer during intermediate sputtering cycles for generation of spectra with high mass resolution and mass accuracy from the same sample area in a single experiment.

First application data including high resolution SIMS spectrometry, MS/MS analyses, high resolution imaging of tissues and depth profiles of biological samples with this new instrument will be presented. For example, single beam depth profiling data were collected, from porcine skin samples, that clearly exhibited different molecular ion signals for different skin layers. This method potentially allows one to measure the permeation of skin for various compounds, e.g. drug molecules.

[1] The 3D OrbiSIMS – Label-Free Metabolic Imaging with Sub-cellular Lateral Resolution and High Mass Resolving Power, Passarelli et al., Nature Methods, 2017, 14(12):1175-1183, DOI 10.1038/nmeth.4504

11:00am **BI-TuM10 Latest Developments in Cluster Beam Technology for ToF SIMS: Towards Greater Spatial Resolution, Improved Ion Yields, and Faster Etch Rates!***, **Paul Blenkinsopp**,* Ionoptika Ltd, UK

The emergence of Gas Cluster Ion Beams (GCIB) for SIMS has significantly extended the available mass range of the technique, and in so doing has also widened the scope of its applications. The low-damage nature of GCIB sputtering greatly improves yields of higher mass molecular species, however spatial resolution remains a challenge. Of particular interest is the ability to detect and image intact bio-molecules in tissue and cells at spatial resolutions below 1 micron. Here, we present on the latest advancements in gas cluster beam technology for ToF SIMS, demonstrating significant progress towards these goals.

We report on the results of our latest innovation – a 70kV Gas Cluster Ion Beam, the GCIB SM. Most current GCIB sources operate at energies between 10 and 40kV, however there are several theoretical benefits to extending the energy range beyond this, including improved focusing and greater secondary ion yields. With the GCIB SM, we demonstrate both a reduction in spot size – improving the resolving power by a factor of 3 – as well as an increase in total current – which has benefits for both speed of analysis, as well as for greater depth profiling capabilities.

We also present on methods to increase ion yields of high-mass species by utilizing alternative source gases to argon. We demonstrate that by choosing an appropriate source gas, yield enhancements greater than 10 times can be achieved. As analysis volumes decrease with greater resolving power, techniques such as this are expected to play a vital role in obtaining the highest quality imaging SIMS data. These latest developments are now enabling SIMS imaging of species such as lipids and gangliosides in tissue at resolutions greater than 2 microns.

11:20am **BI-TuM11 SIMS with Higher Resolution and Higher Signal: 40keV Water Cluster Primary Ion Beam and Prospective Orbital Ion Trapping***, J. Hood, **Peter Cumpson**, I. Fletcher,* Newcastle University, UK*; S. Sheraz,* Ionoptika Ltd, UK

Increasing the secondary ion yield from organic and biological molecules has been a key pursuit in the development of secondary ion mass spectrometry (SIMS) since the inception of the technique, with novel primary ion sources a promising avenue of research. The development of a water cluster primary ion beam has offered improvement in this regard, with ion yield enhancements of the order of 100 to 1000 times observed for beams with water cluster size 7,000, relative to argon cluster beams of size 2,000 [1] [2].

We demonstrate that exploiting larger cluster sizes, in excess of $(H_2O)_{10,000}^+$, with higher beam energy of 40 keV, offers further enhancement of the secondary ion yield, including for large fragments.

To complement the increased secondary ion yield of higher mass fragments, higher mass resolution is desirable. One way to achieve this is through the coupling of a high resolution Fourier transform mass spectrometer (FT-MS) to a SIMS instrument. One form of such hybrid instrumentation utilizes an orbital trapping mass analyser [3] [4], which we have designed and fabricated for our J105 SIMS instrument [3]. However, as with ion cyclotron resonance (FT-ICR MS) techniques, orbital trapping analysers operate at a much slower repetition rate than time-of-flight (ToF) variants, with acquisition dwell times per pixel of the order of 100ms to several seconds, as opposed to as little as 10µs for modern ToFSIMS instruments such as the Ionoptika J105[5].

In FT-MS the field which governs ion motion can potentially be manipulated by applying different voltages to the component electrodes, a process known in ICR-MS as Stored Waveform Inverse Fourier Transform (SWIFT)[6]. The time-domain excitation waveform is formed from the inverse Fourier transform of the appropriate frequency-domain excitation spectrum, which is chosen to excite the resonance frequencies of selected ions. The application of a SWIFT signal to the orbital ion trap improves the speed of acquisition, making high mass resolution SIMS practical.

The combination of a water cluster primary ion beam with high mass resolution orbital ion trapping offers considerable potential for analyzing the molecular chemistry in organic and biological systems.

References

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[2] S Sheraz Nee Rabbani et al. *Analytical Chemistry* 87(4), 2015, 2367–2374

[3] JC Hood et al. *Int. J. of Modern Engineering Research* 6(10), 2016, 76–83

[4] A Pirkl et al. *Microscopy and Microanalysis* 22(Suppl 3), 2016, 340–341

[5] JS Fletcher et al. *Analytical Chemistry* 80(23), 2008, 9058–9064

[6] RB Cody et al. *Rapid Communications in Mass Spectrometry* 1(6), 1987, 99–102

11:40am **BI-TuM12 In-Situ TEM Studies of Biomineralization***, **Tolou Shokuhfar**, R. Shahbazian-Yassar,* University of Illinois at Chicago

This talk will provide an overview of the PIs' efforts to understand the dynamics of biomineralization via in-situ transmission electron microscopy. First we demonstrate how to utilize graphene sheets to build a liquid-cell nanoreactor that fits the chamber of high-resolution TEM. Graphene is impermeable to liquids such as aqueous solutions and therefore can be used to seal liquid solutions from leaking to the high vacuum of TEM environment. In addition, the excellent electrical conductivity of graphene and its ability to scavenge the radicals produced by the interaction of electron beam and liquid solutions provide an excellent platform to perform imaging of biological or hydrated specimens. We then demonstrate our success to observe the biomineralization of calcium oxalate crystals that are the primary constituent of kidney stones. We show that the addition of citrate and other molecular inhibitors can affect the crystallization pathway of these minerals. In addition, we will showcase example of biomineralization of iron oxide core in ferritin proteins and demonstrate the ability to monitor the biomineralization of these crystals using graphene liquid cells in TEM. We will show that the ratio of L and H subunits in the ferritin protein shells can affect the nucleation and growth of iron oxide cores. We also will present our latest results on the biomineralization of magnetosomes in magnetotactic bacteria grown in iron-rich media using in situ GLC-TEM studies.

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