SIMS Solutions in Materials and Life Sciences
Room Great Lakes B - Session SS+BS+FM-ThA3

High Resolution and MS/MS Methods III

Moderator: Gregory Fisher, Physical Electronics USA

4:20pm **SS+BS+FM-ThA3-15 A Fine Analysis of the Composition of Organic-inorganic Complex Layers of Cross-sections from Old Paintings by TOF-SIMS Imaging, Enlightened by MS/MS and Orbitrap***, **Alain Brunelle**, C. Bouvier,* LAMS, Sorbonne Université, CNRS, France*; S. Kayser, A. Pirkl, E. Niehuis,* IONTOF GmbH, Germany*; P. Walter,* LAMS, Sorbonne Université, CNRS, France

When painting, an artist works with diverse mixtures of pigments and binders, which he layers in expert gestures to achieve the intended rendering. The mastery of the binder properties allows him to exploit their possible optical effects, with layers which can be down to a few micrometers thick. Ancient paintings are several centuries old, during which the various heterogeneous mixture of inorganic and organic compounds have interacted with each other and with their environment. Sub-millimeter scale cross-sections of the painting, removed with a blade, offer simultaneous access to all the layers. Such samples are fragile, unique and should be kept as intact as possible to allow subsequent examinations using different analytical techniques.

TOF-SIMS imaging enables the localization and identification of both pigments and organic materials, providing submicron indications of their nature, origin, or state of preservation.[1] With a TOF-SIMS IV instrument, a spatial resolution down to 400 nm can be reached, while preserving mass resolution, in the so-called delayed extraction mode.[2] The identification of binders can nevertheless be ambiguous, since organic materials detected may also be degradation products or surface contaminants. Local optimization of the analysis parameters on small analyzed areas and knowledge of the expected characteristic ions for each binder type proved to be of further importance to avoid erroneous conclusions.[3] Moreover, additional analyses using much more powerful instruments, namely M6 with tandem MS analysis and M6 Hybrid SIMS with Orbitrap™ analyzer, can remove many ambiguities. This is particularly the case when high mass resolution and accuracy are required in conjunction with maintaining lateral resolution in the micrometer range.

Examples will be given with the compositions of fine layers in ancient paintings analyzed in this way. On the one hand, the analysis of samples from the *Infant Bacchanals* (Nicolas Poussin, 1626, Museo Nazionale d'Arte Antica, Rome) has shown that a fine surface layer alters the final appearance of the painting, making it look like a tempera painting, when in fact it is an oil painting underneath. On the other hand, analyses with the modern M6 instruments of cross-sections from the *Ecce Homo* (Titian, 1547, Museo del Prado, Madrid) have provided evidence of a fine inner organic layer, likely made of egg white, and have also shown the presence of a red lake pigment.

References

1 Bouvier *et al*. (2022) doi: 10.1002/jms.4803

2 Vanbellingen *et al*. (2015) doi: 10.102/rcm.7210

3 Bouvier *et al*. (2021) doi: 10.1021.acs.analchem.0c04471

4:40pm **SS+BS+FM-ThA3-17 How Do Water Clusters Work? Insight from Molecular Dynamics Simulations***, M. Kański, S. Hrabar, C. Chang,* ***Zbigniew Postawa,*** Jagiellonian University, Poland

The introduction of water clusters $(H_2O)_n$ to SIMS opened new possibilities in analyzing biological samples. The main advantage of the water clusters is an increase in ion yield by more than an order of magnitude compared to argon clusters of similar size and kinetic energy. The mechanism behind this effect is unknown, though. It has been theorized that a semi-aqueous environment is created in the impact site, which would promote ion creation [1].

We performed molecular dynamics (MD) computer simulations to study the behavior of water clusters that impact the surface of trehalose. Four water clusters consisting of 4000, 7000, 10000, and 25000 molecules had been chosen. The total kinetic energy of each projectile was the same, equal to 20 keV, so the results of the simulations could be compared with the experimental observations [1]. We observed three different projectile behaviors depending on their size (or kinetic energy per molecule). The $(H_2O)_{4000}$ cluster fragments into individual molecules during impact, as do argon clusters of similar size. Decreasing the kinetic energy per molecule (or increasing cluster size) leads to the emission of trehalose molecules enveloped in a partial water shell. The largest projectile bounces from the surface while dissolving trehalose molecules in it. During the presentation, we will discuss the importance of this shifting behavior. Finally, we will show that the amount of emitted water-trehalose complexes correlates with the ion yield observed experimentally.

[1] S. Sheraz (née Rabbani) et al., Anal. Chem. 2019, 91, 9058−9068

The work has been supported by Polish National Science Center Grants 2019/33/B/ST4/01778. Computer simulations were performed on the PLGrid Supercomputer infrastructure and at Penn State's ICDS supercomputer system.

5:00pm **SS+BS+FM-ThA3-19 *In situ* identification, imaging and depth profiling of proteins using 3D OrbiSIMS***, **David Scurr,*** School of Pharmacy, The University of Nottingham, UK **INVITED**

In situ identification of proteins at surfaces has potential applications in areas crucial to health, medicine and medical device development, however, it commonly requires digestion and/or matrix application prior to mass spectrometry. Secondary ion mass spectrometry (SIMS) can potentially overcome these limitations but the analysis of proteins has previously been limited due to fragmentation resulting in only single amino acid secondary ions, devoid of primary structural information.

Employing a gas cluster ion beam (GCIB) moderates fragmentation, resulting in multi amino acid fragments in peptide spectra and molecular ions from proteins up to 12 kDa, however, this method has not been successfully applied for larger proteins. Here we use the 3D OrbiSIMS technique which combines a GCIB and an Orbitrap™ analyser, to achieve *in situ* label and matrix-free 3D mapping of undigested proteins at surfaces. We successfully applied *de novo* sequencing for identification of proteins using fragments generated by the GCIB. We analysed 16 model protein films in a range of sizes from insulin (6 kDa) to fibronectin (272 kDa), achieving amino acid sequence coverages up to 53%. The obtained spectra contain b and y ions, common to low energy collision induced ionisation (CID) and a, c and z ions characteristic to other methods of ionization such as electron capture dissociation (ECD). Similarly, ions observed in negative polarity 3D OrbiSIMS spectra are deprotonated N terminus a, b, c ions and deprotonated C terminus y, z-H and x ions. The 3D OrbiSIMS imaging capability was demonstrated by masking a protein film with a transmission electron microscopy grid, achieving lateral resolution of 10 µm. Additionally we assigned highly specific protein ions in a monolayer biochip sample. Finally, we successfully assigned characteristic peptide sequences from collagen, keratin and corneodesmosin within the depth profile through human skin.

These findings demonstrate a breakthrough approach employing 3D OrbiSIMS to identify proteins by direct surface analysis with minimal manipulation of sample [1].

[1]. Kotowska et al., *Nature Communications*, **11** (1), 2020

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