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Spatial characterisation of immobilised biomolecules on surfaces

Keywords
MALDI-mass spectrometry, biosurface, XPS image, selected area spectroscopy

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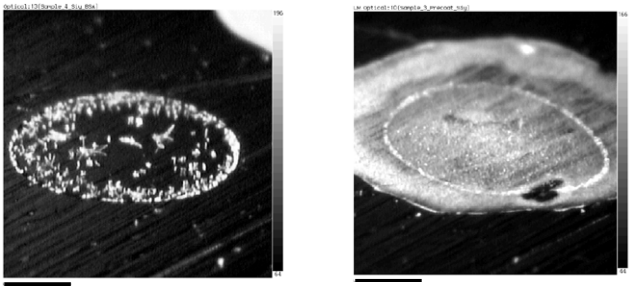
Overview

Analysis of biomolecules on surfaces is essential to various applications of biosensors and biomolecule engineering. Matrix-assisted laser desorption/ionisation (MALDI) is now an established technique for mass spectrometry of biomolecules. Different matrix-analyte preparation protocols have been shown to influence the desorption or ablation process resulting in either high or low metastable fragmentation. It has been speculated that following laser ablation the velocities of the analyte and matrix can be regarded as a valuable and meaningful characteristic of the MALDI process. However, the interaction and distribution of the analyte with respect to the matrix is poorly understood. Here we study the distribution of a selection of biomolecules as a function of matrix material using high resolution imaging X-ray photoelectron spectroscopy (XPS).

MALDI Mass Spectrometry Technique & sample preparation

Samples for Matrix Assisted Laser Desorption Ionisation (MALDI) mass spectrometery are prepared by mixing the analyte with a matrix. The laser energy is absorbed by the matrix and transferred to the analyte, initiating the desorption process with the analyte molecules/ions entering the gas phase in an expanding plume of material. Various mechanisms yield a high number of ionised species which can be separated and detected by the mass spectrometer, although these mechanisms are poorly understood. Furthermore preparation of the sample prior to the MALDI experiment can have a very significant influence on the results obtained. Two common sample preparation techniques used include the 'mixing' technique whereby the analyte and matrix are mixed whilst still in solution on the sample plate or 'pre-coating' the sample plate with matrix and then depositing the analyte onto the dried matrix. The drying method and type of solvents used affects the crystal size and thus the MALDI-MS results with smaller crystals usually yielding more consistent results.



500µm
'mixing' technique

500µm
Pre-coat, then mixing

Figure 1: Optical image of MALDI-MS sample targets prepared by two different techniques.

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XPS from a model MALDI sample

The matrix material used is typically a simple aromatic organic molecule such as DHB or sinapinic acid. Figure 2 shows a survey spectrum and C 1s narrow region spectrum from these two common matrix materials.

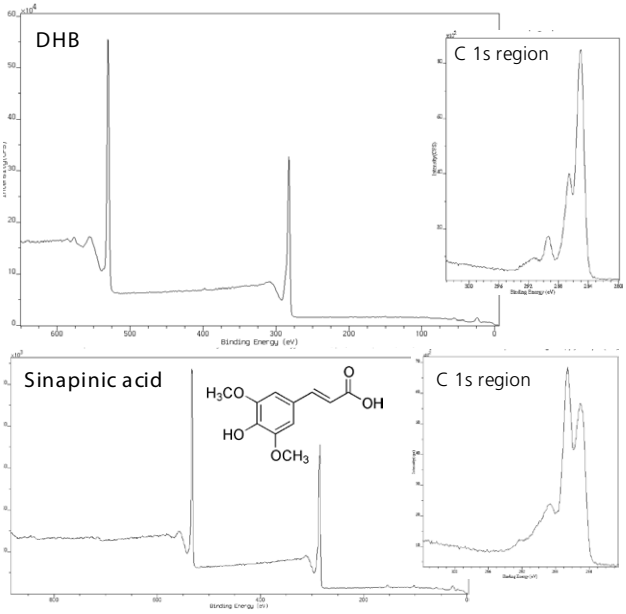

Figure 2: Survey spectrum and high resolution C 1s spectrum from DHB and sinapinic acid.

For this study the analyte chosen is pure bovine serum albumin (BSA) which has both nitrogen and sulphur that is not present in either of the matrix materials. Figure 3 the survey spectrum and C 1s high resolution spectrum from crystalline BSA In this model system the nitrogen signal is used as the marker for the BSA analyte.

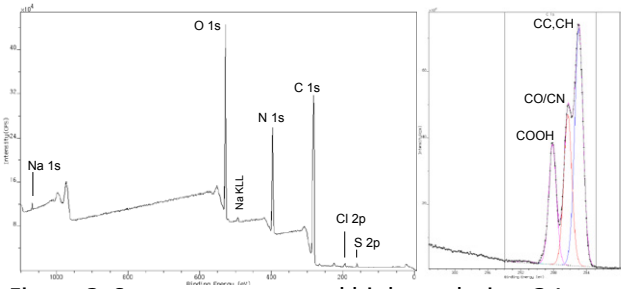

Figure 3: Survey spectrum and high resolution C 1s spectrum from crystalline BSA.

A dilution series experiment was performed for samples prepared by the 'mixing technique' which showed that the N 1s and S 2p could still be detected to a concentration of 0.018 pmol similar to concentrations used for MALDI sample preparation. Subsequently an analyte-matrix sample was prepared using the 'pre-coat' method outlined in figure 4.

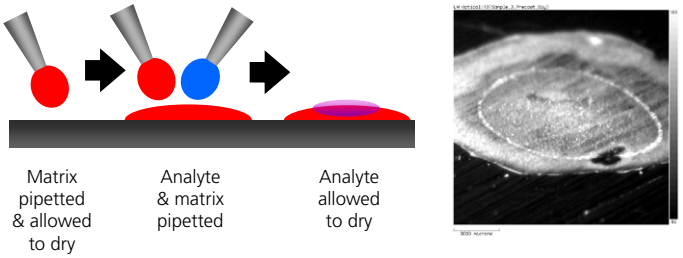

Figure 4: A schematic diagram outlining the 'pre-coat' method where the matrix-analyte are mixed on a dried layer of matrix. The resultant sample is shown in the optical image.

The lateral distribution of elements within the sample was determined using XPS elemental imaging. A high resolution stitched image was acquired over a 3.2 mm x 3.2 mm area shown in Figure 5 below. The localised nature of the nitrogen from the analyte (green) relative to the matrix and Ni (red) of the sample platen is easily observed. The single Ni image shown on the right of Figure 5 shows the regions of interest adjacent to the defect observed in the optical image.

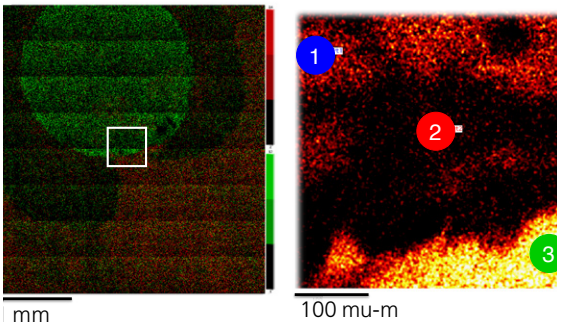

Figure 5: Overlaid 8x8 stitched image of Ni (red) and nitrogen (green) and a single Ni image used to define the position for multi-point, selected area XPS.

Spectra were acquired from 55 µm diameter selected areas defined from the low magnification parallel image. Three areas of interest were identified adjacent to the defect region observed in the optical image. The defect area (3) is shown to have no coverage of matrix or analyte material and shows a spectrum characteristic of the stainless steel sample bar. The area directly adjacent to the defect region identified by the dark area in the right hand image of Figure 5 is characteristic of a survey spectrum of the BSA analyte and indicates that the matrix/analyte completely attenuates the spectrum from the substrate sample bar. The spectrum from this area is shown as spectrum (2) in Figure 6. Spectrum (1) is characteristic of a thin overlayer of BSA on the sample bar.

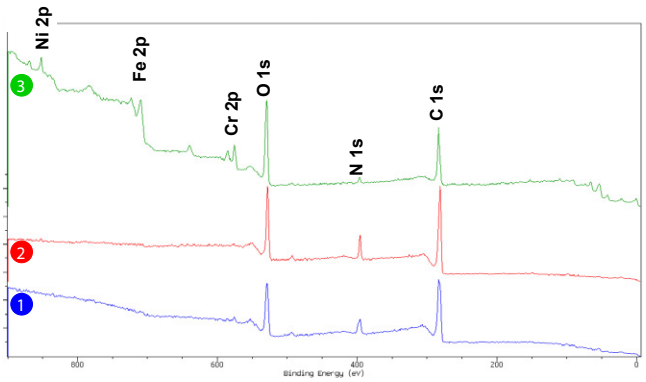

Figure 6: 55um diameter selected area spectra acquired from 3 regions of interest defined from the Ni image shown in Figure 5.

Conclusion

It has been demonstrated the different analyte-matrix preparation protocols affects MALDI spectra.

The distribution of a protein in matrix has been investigated and reported.

The pre-coat method gives much more uniform distribution of the matrix-analyte across the spot, with some evidence of surface segregation of the protein.

Further understanding of surface chemistry holds the key to improving matrix distribution.

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