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Supplementary Material

Effect of Structured Surfaces on MALDI Analyte Peak Intensities

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Experimental

Materials and Reagents

alpha-cyano- 4-hydroxy cinnamic acid (aCCa) and the peptide calibrants were purchased from Sigma-Aldrich (Dorset, UK). Insulin was obtained from Fluka (Dorset, England). Dextran was either dissolved in distilled water or 70 % acetone / 30 % water and used initially to calibrate the mass spectrometer. All solvents were analytical grade and were purchased from Sigma-Aldrich.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) mass spectrometry.

The matrix-assisted-laser-desorption / ionization was conducted on a Bruker Biflex (Bruker, Coventry, U.K), with a 20 kV extraction with 10 kV post-acceleration, using a nitrogen laser at 337 nm. Positive (and negative ion) mode spectra in the linear mode were sampled with a sum of twenty shot spectra unless otherwise noted.

MALDI-TOF Calibration

The spectra were calibrated with the $[M+H]^+$ ion of substance P (1348.67 Da), bradykinin (1061.24 Da), bovine insulin (5734.56 Da) and the matrix. Although no external sodium chloride

was added, the spectra indicated that the peaks were sodiated and no difference was observed by addition of sodium, except for dextrans, where the sodiated peak intensity increased between 5-20 % compared to the sodiated species in experiments where no supplemental sodium chloride was added (data not shown). In both ionization techniques, these ions formed the main distributions of dextrans in the negative-ion mode. It negative ion mode, the main species was the fragmented byproduct from $\alpha 1 \rightarrow 6$ dissociations to produce the $[M - H - 120]^-$ species.

Surface Modification Procedure

To demonstrate whether surface treatment could result in enhancement of analyte peak intensity, all experiments were repeated a number of times (n = 10) with the same matrix (alpha-cyano- 4-hydroxy cinnamic acid) [M] and analyte (substance P, m/z 1347.74) [A], salt (NaCl) [S] and same e-irradiation time (20 KeV for 30 seconds in a standard scanning electron microscope, SEM, Jeol, Department of Physics) [I]. Threshold laser power was utilized and the surface was modified by physical or chemical means. A number of surface modifications were employed and compared to where the surface was not modified ('no treatment') [NT]. The treatments were sandblasting [SB]. Here, the metal surface was modified using 400 grit then 800 grit sand paper alternating the direction with each grit, using Dremel with ½ inch felt wheels. For 'chemical etching' [CE], the surface was washed with deionized water and dried with cotton paper. To the dried surface a strong acid (0.1 M hydrofluoric acid) was applied for 1 h and surface washed thrice with deionized water. The surface was then dried using a cotton tissue. The process was repeated three times. For generating a surface that was'shined chemically' [SC]), the stainless steel metal surface was polished using non-waxy aluminum oxide based polish. The surface was immersed in deionized water with 0.1 % hydrochloric acid for 4 h. The surface was rinsed with deionized water three times and dried with a cotton towel. A few drops of mineral oil was applied to the surface and using a cotton tissue, the surface was rubbed until the surface shiny and reflective. The oil was re-applied four times and the rubbing procedure repeated until the surface showed a mirror-like shine. On the unmodified or modified surface either the salt (0.25 M sodium chloride in deionized water) [S] was deposited, or matrix (0.1 M cyano- 4-hydroxy cinnamic in 1:1 water: acetonitrile with 0.01% trifluoroacetic acid) [M] and finally the analyte (100 μM substance P in 1:1 water: acetonitrile with 0.01% trifluoroacetic acid) [A]. In between the deposition of salt or matrix or analyte, the surface was irradiated [I] with an electron beam to

promote surface deformations. The order of layering and the position of irradiation was abbreviated using two letter code followed by one letter code to denote the various components.

In figure 1, the matrix to analyte ratio between aCCa:SP was 2000:1. A number of shots per spectra, m=20, the number of samples per treatment, *n* was 10. Matrix suppression mode was not utilized.
For figure 2, the number of shots per spectra, *m* was 20, the number of samples per treatment (and *n* was also 10) and the matrix to analyte ratio between aCCa:SP was 2000:1. The error bars were removed for clarity.
The Newman-Keuls multiple comparisons test at the 0.05 significance level, show SCMIA versus SBSMA, SBIMIA, NTSMA, CESMIA, CESIMIA, SBSMIA, SCIMIA and SCSIMIA and the reverse combination was significant for the SP protonated species (q=2.8 – 4.4). For the sodiated species, three treatments were significant. These were SCSMIA, SCSIMIA, and CESIMIA. These treatments were significant against SBSMA, NTSMA, SBSMIA, SBSIMIA, CESMIA, CESIMIA, and SCSIMIA (q= 3.3 – 4.4) and a single treatment (CESMIA versus SBSMA, q=3.9) as well as the reverse combinations. A separate analysis of laser power showed all combinations were significant against each other for the pooled positive-ion species.
For figure 3, the matrix to analyte ratio between aCCa:SP was 2000:1. A number of shots per spectra, *m* was 20, the number of samples per treatment, *n* was 10. The Newman-Keuls multiple comparisons test at the 0.05 significance level, show NT versus SC is significant for the SP sodiated species (q=3.8). Also, the NT LP was significant against SC and SB as well as the reverse combinations.