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A NOVEL APPROACH TO MALDI-TOF-MS SAMPLE PREPARATION

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Introduction

MALDI-TOF-MS has become a well established technique for the analysis of biological samples. This has been mainly due to its ease of use and relative insensitivity to biological matrixes which are used in the preparation of most biological samples. However, it has been previously demonstrated that removing these contaminants can significantly improve the quality of the resulting spectra.

Furthermore MALDI-TOF-MS is now a technique which is routinely automated for both the sample preparation and analysis of many biological samples, which include those resulting from a proteomics experiment. Clearly, any method of sample preparation developed for MALDI-TOF-MS applications must be amenable to full automation.

Presented in this poster is a new plate design for sample preparation of biological samples (patents applied for). The plate design allows for the concentration of very dilute samples and the removal of inorganic salt contamination. The whole sample preparation procedure is performed in full upon the MALDI-TOF-MS sample stage. Presented in the poster is the optimised plate design and preparation methods to significantly improve the quality of the resulting MS spectrum compared with conventional MALDI sample preparation.

The MALDI sample preparation of protein digests resulting from in gel digestion of gel spots from 2D gel electrophoresis are evaluated. It is shown in this poster that the sensitivity of the mass spectrometer is dramatically improved for faint gel spots resulting from both Coomassie blue and silver stained gel pieces. The method is compared with alternative methods of sample preparation and its amenability for automation is demonstrated.

Methods

The sample stage or plate of a MALDI-TOF mass spectrometer has been modified such that an increased sample volumes (upto 10 µL rather than typically 1 µL) can be loaded. The increased volume is allowed to focus onto a small region of an adsorbent surface.

In addition, the deposited sample containing bio-molecules such as peptides can be washed in situ with water to remove contaminants such as inorganic low molecular salts (for example NaCl), detergents (for example SDS and CHAPS), organic buffers (for example TRIS). Therefore the resulting mass spectra are significantly enhanced by the focussing and clean-up of the sample. The sample preparation process is a simple procedure and is amenable to full automation by a liquid handling robot (MassPREP Micromass).

The standard stainless steel MALDI sample plate (**Figure 1a**) is coated with a thin layer of PTFE film. The centre of the sample well is then etched to remove the PTFE film to produce a small clearing with a diameter of 0.4-0.6 mm (**Figure 1b**). The entire plate surface is then coated with a thin film of a hydrophobic polymer that has adsorbent properties (**Figure 2**).

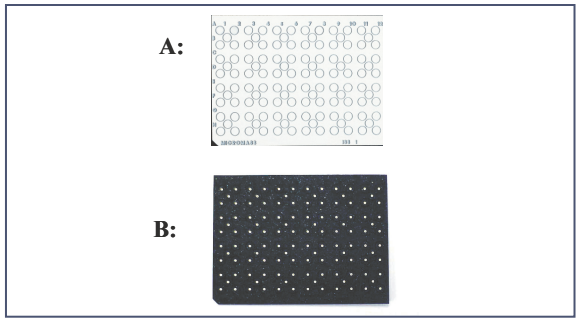

Figure 1. The MALDI-TOF-MS sample stage (a) The standard stainless steel plate (b) The modified MALDI sample stage

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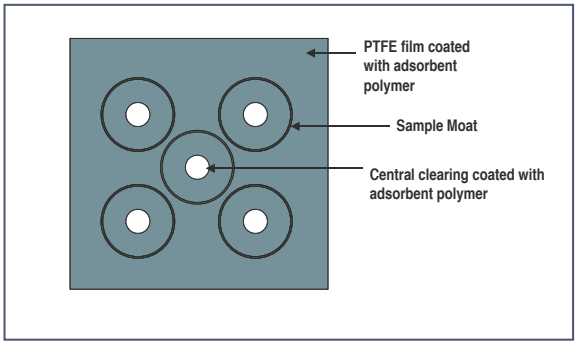

Figure 2a. An aerial view of the modified target plate

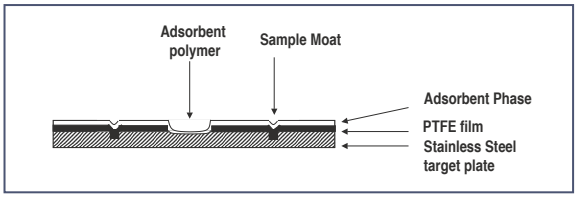

Figure 2b. A cross sectional view of the modified target plate

The sample preparation method is described as follows. The adsorbent phase surface is activated by adding a small volume (approximately 1µL) of an organic solvent such as acetonitrile. The organic solvent allows the hydrophobic adsorbent phase to be wetted. An equal volume of water is then added to the organic solvent to reduce the rate of evaporation. This conditioning solution is then removed just prior to the addition of sample. The sample is deposited in a large volume of 5-10µL. The sample solution contains 20-30% acetonitrile to further aid wetting of the adsorbant phase surface. The large volume sample loading is possible because the hydrophobic surface provides an increased contact angle with the sample solution compared to the stainless steal sample plate. In addition the sample moat geometry maintains the high contact angle and acts as a barrier to the droplet perimeter. The combination of both the hydrophobic surface and sample moat gives an approximate 5-10 fold improvement in sample volume retention (**Figure 3**).

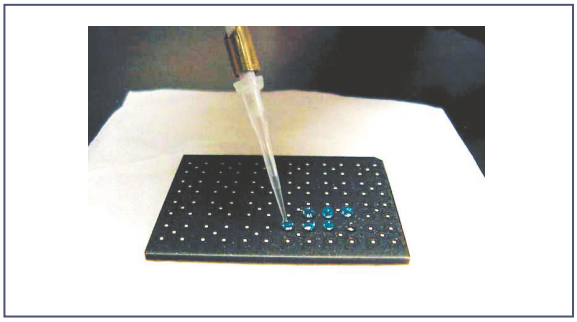

Figure 3. The sample volume capacity on the modified target plate

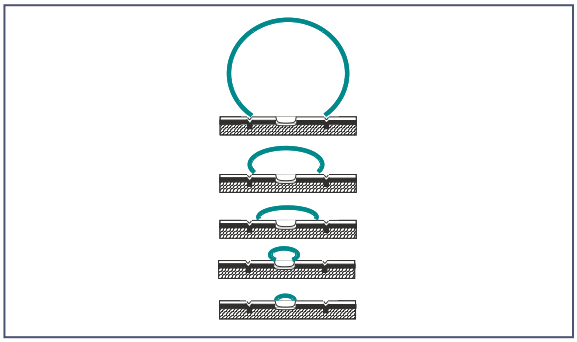

Figure 4. Focussing and sample localisation of target plate macro-structure

The analyte solution is allowed to evaporate. During the evaporation the analyte bio-molecules are immobilised onto the central portion of the adsorbent phase surface. The bio-molecules are forced to the surface by non-specific hydrophobic interactions. Although the adsorbent phase polymer is highly hydrophobic, significant focussing of the sample to the central portion of the adsorbent phase surface is observed (**Figure 4**). Once the sample solution has completely evaporated the analyte bio-molecules become immobilised to the adsorbent phase surface. The sample positions can then be washed individually with the use of a pipette to remove the inorganic salts (**Figure 3**).

The washed sample can then be analysed directly on the sample plate by the addition of a small volume of MALDI matrix such as CHCA in approximately 1µL. The matrix solvent has a high organic content typically 70-90%.

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The solvent will interact with the bio-molecules from the adsorbent phase surface so allowing the co-crystallisation of analyte and matrix whilst being focussed into a small area. The sample plates were analysed by MALDI-TOF-MS (M@LDI Micromass)

Results

Resolution

A solution of Alcohol dehydrogenase (ADH; 100 fmol) was deposited onto the modified target plate. The sample was washed in TFA (0.1% v/v) before the addition of MALDI matrix CHCA (0.1 mg/ml). The sample was then analysed by MALDI-TOF-MS and the full width at half maximum resolution was estimated from the $(M+H)^+$ 968 and 2212 ions (**Figure 5**). The FWHM resolution of 13000 and 13500 is typical for the routine resolution obtained from the standard MALDI-TOF-MS target plate (data not shown)

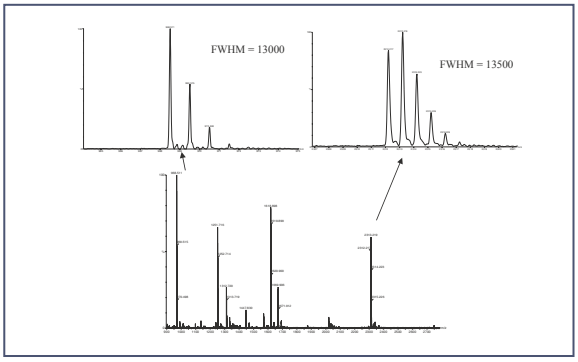

Figure 5. MS resolution on the modified target plate

Mass Accuracy

The mass measurement accuracy was investigated on the surface of the modified plate. A solution digested of ADH was deposited onto the target plate at four concentrations (1, 10, 100 and 1000 fmol) and a calibrant was added to the near point sample well at a concentration of 100 fmol. The sample wells were analysed by MALDI-TOF-MS and the resulting spectra were lock mass corrected twice, once from a single internal peptide and once from the near point calibrant peptide.

The mass measurement accuracy can be seen in **Figure 6** are the overall RMS mass measurement accuracy for the internal and near point method are 7 and 37, respectively.

Sample Conc	1 pmol					
Well No	A1	A5	A9	B1	B5	B9
Internal (PPM)	3	5	7	6	5	11
Near point (PPM)	3	25	17	12	20	71
Sample Conc	0.1 pmol					
Well No	A2	A6	A10	B2	B6	B10
Internal (PPM)	4	6	9	3	8	8
Near point (PPM)	28	18	31	7	45	43
Sample Conc	0.01 pmol					
Well No	A3	A7	A11	B3	B7	B11
Internal (PPM)	3	11	6	7	7	7
Near point (PPM)	29	60	34	42	16	40
Sample Conc	0.001 pmol					
Well No	A4	A8	A12	B4	B8	B12
Internal (PPM)	5	8	10	7	11	11
Near point (PPM)	44	36	60	18	30	47
RMS (PPM)	Near point 32				Internal 7	

Figure 6. Mass measurement accuracy from the modified target plate

Sensitivity and Sample Focussing

The sample focussing effects and limit of detection of the modified target plate were demonstrated from a solution digest of BSA. A serial dilution of intact BSA was made to give two solutions at 1fmol and 0.2fmol prior to digestion. After digestion with trypsin equal quantities of digest sample were deposited onto the plate (1 µl of 1fmol/µl and 5µl of 0.2fmol/µl). The samples were washed before analysis by MALDI-TOF-MS. The resulting spectra are shown in **Figure 7**.

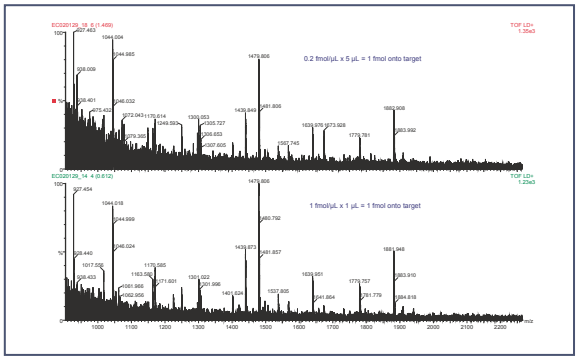

Figure 7. The Sensitivity and Sample Focussing of the Modified Target Plate

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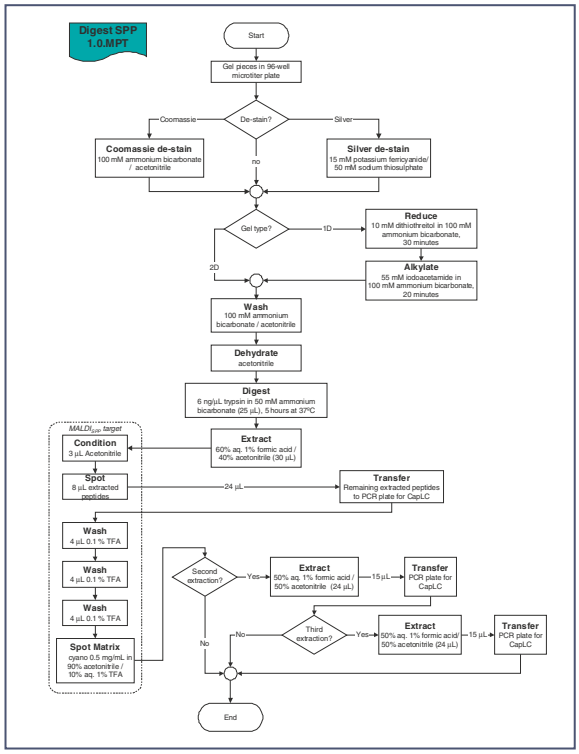

Figure 8. A flow diagram summarising the automate protocol for in gel digestion and sample deposition onto the modified target plate

Salt tolerance

The modified target plate was demonstrated to facilitate the successful removal of a range of common contaminants. Good quality spectra could be obtained after washing from samples contaminate with the following salts and detergents (NaCl 250mM; SDS, 1.0%; Tris, 25mM; Glycine, 100mM). After washing no significant difference could be observed between the contaminated and non-contaminated spectra (data not shown)

Automation

The standard digestion protocol of the liquid handling system (MassPREP Station; Micromass) was modified to incorporate sample deposition and washing on the modified target plate. A flow diagram summarising the modified protocol can be seen in **Figure 8**.

Analysis of sample from SDS-gel electrophoresis Samples of BSA (250 fmol) were ran on a 1 D SDS-PAGE (Bio-Rad). The gel was stained with Coomassie Brilliant Blue (Bio-Rad) and bands at approximate 70 KDa were excised and place in a 96 well microtitre plate. The resulting gel pieces was automatically destained, reduced and alkylated and then digested with trypsin use the robotic liquid handling system (MassPrep: Micromass UK Ltd). An aliquot (2µL) of the extracted peptides was deposited onto a standard stainless steel MALDI target plate before the remaining extracted peptide (6µL) were deposited and wash on the modified target plate. The two plates were analysed by MALDI-TOF-MS (**Figure 9**). The resulting spectrum from the modified target plate shows an improved signal to noise and peptide coverage compared to the standard spotting.

A second set of samples of BSA (250 fmol) were ran a SDS-PAGE and were stained with silver stain (Bio-Rad) and bands were excised and digested as above. The resulting extracted peptides were prepared on either a modified target plate or prepared using a micro reverse phase pipette tip (ZipTip; Millipore) and spotting to a standard target plate. The resulting MALDI-TOF-MS spectrum from the modified target plate shows an significant improvement in both signal to noise and peptide coverage compared to the standard plate (**Figure 10**).

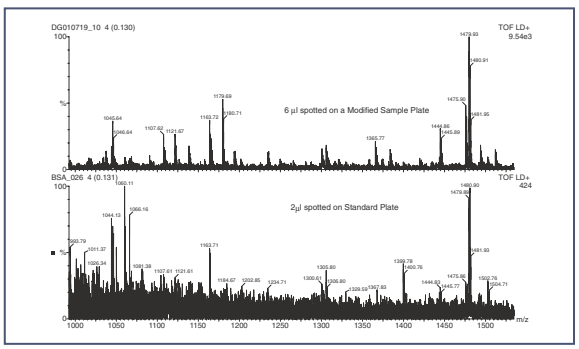

Figure 9. A comparison of the modified target plate and the standard target plate

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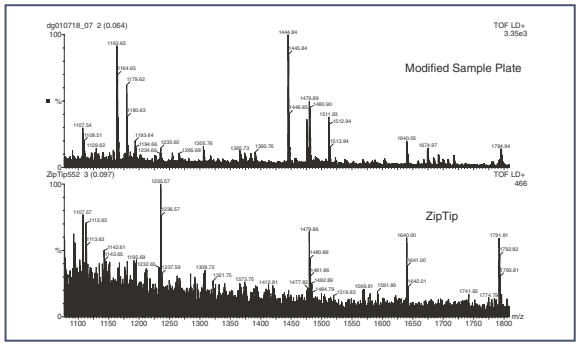

Figure 10. A comparison of the modified target plate and a micro reverse phase pipette tip

Conclusion

- A modified target plate has been developed which allows large volumes (1-10 µL) of sample to be deposited onto the MALDI-TOF-MS sample stage. The modified target plate can be washed to effectively remove sample contaminants. Biomolecules of interest remain attached to the hydrophobic surface.
- The modified target plate does not impede the resolution and mass measurement accuracy of the MALDI-TOF-MS. Furthermore the modified target plate significantly enhances the sensitivity of the MALDI-TOF-MS allowing limits of detection in the atomolar range.
- The deposition and subsequent washing is amenable to automation can be incorporated into a full digestion protocol
- The modified target plate is shown to give significant improvements when analysing samples prepared from an in gel digestion. The peptide coverage and signal-to-noise is significantly enhanced compared with standard preparation methods.

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