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An Integrated Approach to Desalting and Concentrating Peptide/Protein Samples for MALDI-MS Analysis

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Introduction

Matrix-assisted laser desorption and ionization mass spectrometry (MALDI-MS) is a powerful tool for protein identifications. This has been mainly due to its ease of use and relatively insensitivity to biological matrixes resulting from sample preparations. However, it has been demonstrated that the success rate of protein identification can be greatly enhanced by improving the sensitivity of analysis and by removing the contaminants contained in biological samples.

Traditionally, a micro-column packed with reverse-phase materials such as ZipTip™ is often used as a final desalting and concentrating step prior to MALDI analysis.[1] Because micro-columns can process sample as little as submicro-liters, this processing method is well suited to proteomics research where available sample volume for analysis is often very limited. However, as an additional step in sample preparation, desalting of the samples is often time consuming and will lead to sample loss.

Presented in this poster is an innovative sample preparation technology for MALDI analysis of biological samples. It integrates both sample desalting and sample concentrating functions into a MALDI plate, and therefore allows sample preparation directly on the plate, thus considerably simplifying sample preparation process. Samples containing commonly used salts or detergents were evaluated, and the results are compared with alternative MALDI sample preparation method. Furthermore, it is shown that analysis of peptide mixtures (artificial mixture and protein digests) performed by the plate shows excellent sensitivity - typically one order magnitude improvement over standard stainless steel plates.

Experimental

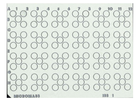
1. Description of the Plate

- On a regular stainless steel plate, a thin layer of Teflon is coated
- A sample well of 0.6 mm is created on the Teflon (**Figure 1**)
- The entire plate surface is coated by a thin film of a hydrophobic polymer that has peptide/protein-binding properties

2. Sample Preparation Method

- Sample solutions are prepared in 30% acetonitrile (v/v), 0.1% TFA (v/v)
- Deposit up to 10 µL of sample (**Figure 2**), wait for sample to dry completely
- Add 5 µL of washing solution (0.1% TFA) and wait 3 min, remove washing solution, and repeat this step two more times
- Wait for sample to dry completely
- Add 1µL matrix solution (CHCA 0.5mg/ml, 70% ACN (v/v) 0.1% TFA (v/v))
- Wait for matrix to dry and analyze by MALDI-TOF MS

A



B

Figure 1. The comparison of standard (A) and modified (B) MALDI sample plate

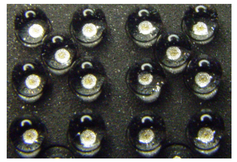


Figure 2. The sample volume capacity on the modified sample plate

Results

1. Removal of Inorganic Salts and Detergents

(A) Removal of 1 M Urea

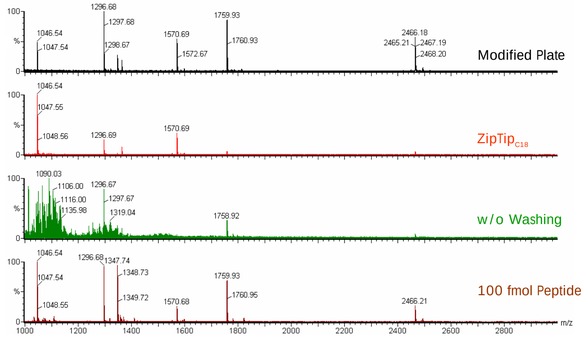


Figure 3. Examples of MALDI spectra from samples processed by the modified plate and ZipTip$_{C18}$. The peptide standard mixture solution (100 fmol/µL) contains 1 M urea.

(B) Removal of 0.1% SDS

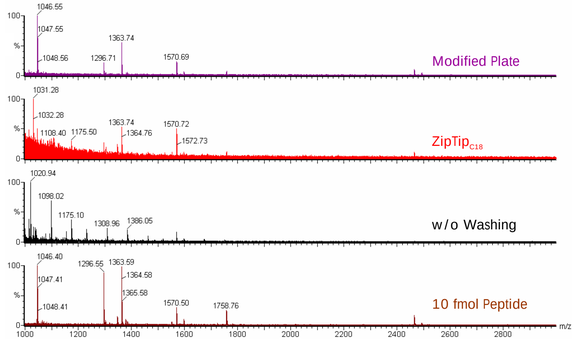


Figure 4. Examples of MALDI spectra from samples processed by the modified plate and ZipTip$_{C18}$. The peptide standard mixture solution (10 fmol/µL) contains 0.1% SDS.

2. Limit of Detection and Sample Focusing

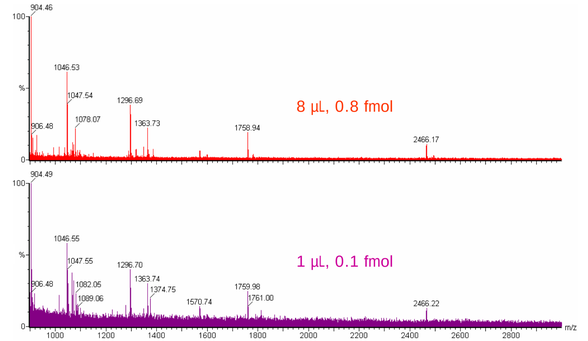


Figure 5. The sample focusing effects and limit of detection of the modified target plate. The concentration of applied sample was 0.1 fmol/µL.

3. Analysis of Samples from In-gel Digestion

Samples of Ovalbumin were run on a 1 D SDS-PAGE (Bio-Rad). The gel was stained with silver stain (Invitrogen). The band at approximate 44 kDa was excised and digested according to a published procedure.[2]

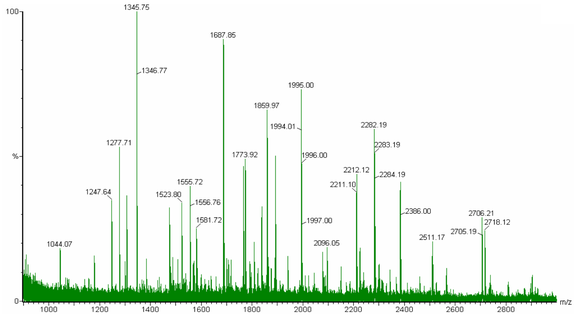


Figure 6. MALDI spectrum of 1µL of in-gel digest of Ovalbumin (~200 fmol) from the modified plate. The sample was washed three times with 0.1% TFA before the addition of matrix solution.

Conclusions

- 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 plate. In addition the sample can be washed to remove common contaminants such as salts and detergents.
- The modified target plate significantly enhances the sensitivity of the MALDI-TOF-MS allowing limits of detection in the sub-femtomole range.
- The modified target plate is robust and very simple to use. The sample preparation time is greatly reduced compare to standard preparation methods. With a robotic liquid handling system, the whole procedure is amenable to automation.

References

1. Gobom, J., Nordhoff, E., Mirgorodskaya, E., Ekman R., and Roepstorff, P. *J. Mass Spectrom.* 34, 105-116 (1999)

2. Shevchenko, A., Wilm, M., Vorm, O. Mann, M. *Anal. Chem.* 68, 850-858 (1996)