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

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## Overview

- A modified MALDI target plate is developed that enables on-target sample cleanup and high-volume sample loadings (up to 10 µL).
- The usage of the plate has greatly reduced sample preparation time and dramatically improved sensitivity of analysis (sub-femtomole).

## Introduction

Biological methods for preparation/purification of peptide/protein samples often result in an overwhelming stoichiometric excess of inorganic salts and detergents that interfere with MALDI analysis. A clean-up step following sample preparation is frequently necessary for a successful MADLI analysis. However, desalting of the samples is often time consuming and leads to sample loss.

Presented in this poster is an innovative sample preparation technology for peptide and protein analysis via MALDI mass spectrometry. The technology utilizes a MALDI plate as a platform, enabling on-target cleanup and high-volume sample loading, considerably simplifying sample preparation process. The poster will demonstrate that contaminated peptide/protein samples can be directly analyzed by a MALDI mass spectrometer after on-target cleanup. Examples with commonly used salts or detergents show that the cleanup does not affect the overall sensitivity and is as least as efficient as other common purification methods. In addition, the design allows the use of an increased sample volume so samples with low concentrations can be focused to a confined region. Analysis of peptide mixtures (artificial mixture and protein digests) performed by the plate shows excellent sensitivity (with at least one order magnitude improvement over standard stainless steel plates). Furthermore, sample deposition and subsequent washing are amenable to automation can be easily incorporated into a full digestion protocol.

## 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, 0.1% TFA (v/v))
- Wait for matrix to dry and analyze by MALDI-TOF MS (Micromass M@LDI-R)

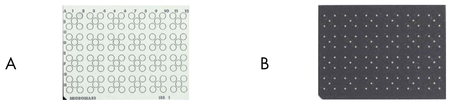


Figure 1. The comparison of standard (A) and modified (B) MALDI sample plate

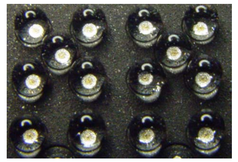

Figure 2. The sample volume capacity on the modified sample plate

## Results

### 1. Removal of Inorganic Salts and Detergents

(A) Removal of 1 M Urea

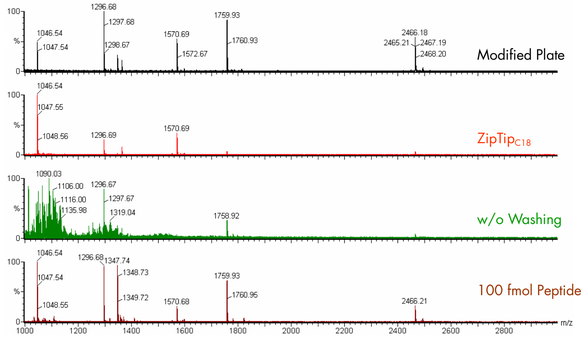


Figure 3. Examples of MALDI spectra from samples processed by the modified plate and ZipTip$_{C18}$. The peptide standard mixture solution (100 fmol/µL) contains 1 M urea.

(B) Removal of 0.1% SDS

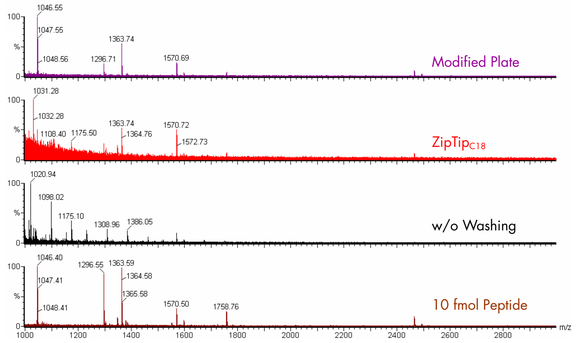


Figure 4. Examples of MALDI spectra from samples processed by the modified plate and ZipTip$_{C18}$. The peptide standard mixture solution (10 fmol/µL) contains 0.1% SDS.

### 2. Limit of Detection and Sample Focusing

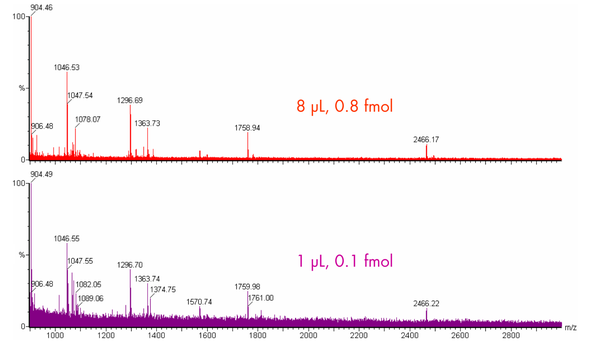


Figure 5. The sample focusing effects and limit of detection of the modified target plate. The concentration of applied sample was 0.1 fmol/µL.

### 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]

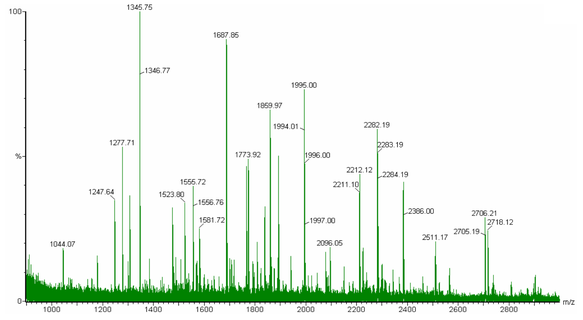


Figure 6. MALDI spectrum of 1µL of in-gel digest of Ovalbumin (~200 fmol) from the modified plate. The sample was washed three times with 0.1% TFA before the addition of matrix solution.

## Conclusions

- A modified target plate has been developed which allows large volumes (1-10 µL) of sample to be deposited onto the MALDI 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)

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