Novel Approach to MALDI-TOF-MS Sample Preparation

Weibin Chen, Peter J. Lee, Asish B. Chakraborty, Santiago Vazquez, Scott Berger, Jeffrey W. Finch, John C. Gebler
Life Sciences R&D, Waters Corporation, Milford, MA 01757

Overview

- A novel MALDI target plate, Mass*PREP*™ PROtarget, was 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

This work presents a simple and robust sample preparation technology for MALDI-MS analysis by using a novel MALDI target plate, Mass*PREP*™ PROtarget. PROtarget is a uniquely designed MALDI target plate that enables sample preparations directly on the plate. A large volume (up to 10 µL) of diluted sample is placed on the target plate and dries down with focusing to a confined region coated by a membrane having peptide binding property. The region is then subsequently washed to remove contaminates that are contained in sample and may interfere with MALDI analysis, whereas analytes are selectively adsorbed onto the membrane and enriched.

The poster will demonstrate that:

- Significant concentration of very dilute sample can be achieved by applying an increased sample volume to Mass*PREP*™ PROtarget .
- Contaminated samples can be directly analyzed by a MALDI mass spectrometer after on-target cleanup, and overall sensitivity is not affected by the cleanup.
- The sample preparation of protein digests coming from in-gel digestion of 2D electrophoresis gel spots has significantly improved the sensitivity.
- Micro-scale chemical reactions can be performed in-situ on the MALDI target plate.

Experimental

1. Description of the Target 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 sample well is then 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, 90% ACN, 0.1% TFA (v/v))

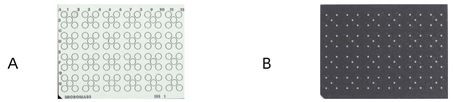


Figure 1. Standard stainless steel plate (A) and Mass*PREP*™ PROtarget (B)

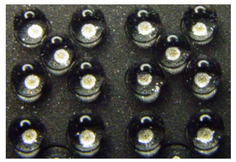


Figure 2. The sample volume capacity on Mass*PREP*™ PROtarget. The volume of each droplet shown in the picture is 10 µL.

Results

1. Limit of Detection and Sample Focusing of Mass*PREP*™ PROtarget

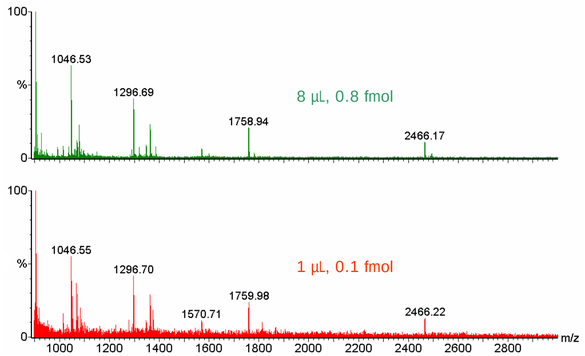


Figure 3. The sample focusing effects and limit of detection of Mass*PREP*™ PROtarget. Different aliquots of the same peptide mixture were added to the target. The concentration of applied sample was 0.1 fmol/µL.

2. Removal of SDS (0.1%) Contained in Peptide Samples

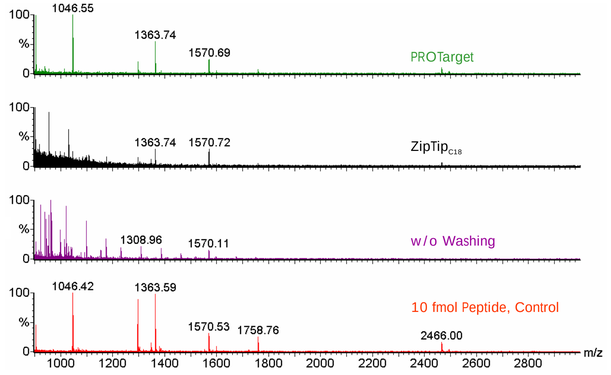


Figure 4. Examples of MALDI spectra from samples processed by Mass*PREP*™ PROtarget and ZipTip$_{C18}$. The peptide standard mixture solution (10 fmol/µL) contains 0.1% SDS.

3. Guanidination and Purification of Tryptic Peptides

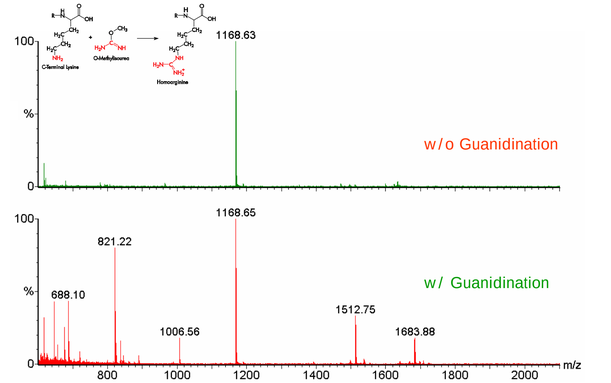
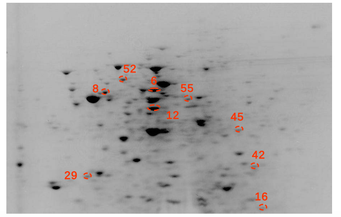


Figure 5. Mass spectra from 300 fmol Cytochrome C tryptic digest without guanidination and with guanidination. The guanidination reaction mixture was prepared according to a published method, and was directly applied to Mass*PREP*™ PROtarget. Upon drying, the sample was washed with 0.1% TFA three times to remove excess amount of *o*-methylisourea (0.5M) in the reaction mixture.

4. Identifications of Unknown Yeast Cytosol Proteins Separated by 2D Gel Electrophoresis



2D Gel Images of Yeast Cytosol.
Gradient Gel: 10-20%, pH: 3—10.

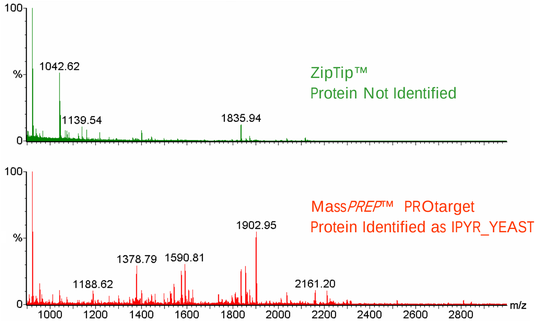


Figure 6. MALDI spectra from one of excised gel spots(#55). A number of spots (circled in the 2D map) were excised and digested using Micromass Mass*PREP*™ station, giving 25 µL of extract. For comparison, same volume (5 µL) of extract was processed, in parallel, using Mass*PREP*™ PROtarget or ZipTip™.

Conclusions

- A novel target plate, Mass*PREP*™ PROtarget, has been developed to allow the whole sample preparation procedure to be directly performed on the MALDI target plate, thus greatly reducing sample losses in the sample preparation process. The unique design of Mass*PREP*™ target allows both desalting and concentrating of applied samples accomplished on the target.
- Mass*PREP*™ PROtarget significantly enhances the sensitivity of the MALDI-TOF-MS analysis allowing limits of detection in the sub-femtomole range.
- Mass*PREP*™ PROtarget is robust and very simple to use. With a robotic liquid handling system, the whole procedure is amenable to automation.
- The peptide/protein-binding surface of Mass*PREP*™ PROtarget can be regenerated for repeated usage.