

Rapid and Efficient Sample Clean Up On Hydrophobic Probe Surface for AP-MALDI Analysis

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OVERVIEW

- Preparation of a C-18 Hydrophobic MALDI target plate.
- On-probe sample clean up on Hydrophobic MALDI target plates to remove contaminants that cause signal suppression.
- Hydrophobic MALDI target plates provided for fast and efficient sample clean up for AP-MALDI MS and MS/MS analysis of biomolecules.

INTRODUCTION

Buffer salts, detergents and chaotropes associated with bio-molecules cause signal suppression in Matrix-assisted laser desorption/ionization mass spectrometry (MALDI).

Traditionally used C-18 chromatographic materials for purification, such as ZipTIPs, and SPE columns, can be expensive and time consuming.

Recently Orlando and co-workers prepared C18 coated MALDI plates and demonstrated on-probe sample clean up for large sample volumes^{1,2}.

We have optimized the conditions for on-probe clean up for small sample volumes (1 μ L) and have demonstrated that this method is more efficient and less time consuming than the ZipTip clean up method.

EXPERIMENTAL

Molecular Biology Grade water was purchased from Biowittaker (Walkersville, MD, USA). α -cyano-4-hydroxycinnamic acid (4-CHCA) was obtained from Fluka (Buch, Switzerland). MALDI matrix solution was prepared as 10 mg/mL solution of 4-CHCA in 60% acetonitrile/0.1% TFA. Immobilized trypsin beads (Poroszyme Bulk Immobilized Trypsin) were purchased from Applied Biosystems (Foster City, CA, USA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Microbial samples:

Escherichia coli bacteriophage MS2 was purchased from ATCC (Manassas, VA) along with the host *Escherichia coli* strain C3000. MS2 bacteriophage was propagated and purified according to the previously published procedure. Spores of *Bacillus globigii* were obtained from US government.

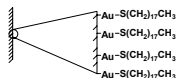
Mass Spectrometry:

All mass spectral experiments were carried out on a Thermo Finnigan (San Jose, CA, USA) LCQ Deca XP ion trap mass spectrometer integrated with an AP/MALDI ion source with pulsed dynamic focusing (MassTech Inc., Columbia, MD, USA) using positive ionization mode.

Preparation of C-18 coated gold plates

1) Incubate plate in Methanol:HCl (1:1) solution. Rinse thoroughly with deionized water and dry.

2) Incubate plate in 2 mM solution of octadecanethiol in absolute ethanol. Wash the plate with excess absolute ethanol to remove residual octadecanethiol.



C18 coated gold plate showing the magnified surface

On probe clean up protocol:

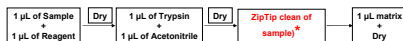
1. Clean up after trypsin digestion



2. Clean up before trypsin digestion



ZipTip clean up method:



ZipTip protocol:

- Wet C18 Ziptip (Millipore) using 50% acetonitrile (2 X 10 μ L)
- Equilibrate with 0.1% TFA (2 X 10 μ L).
- Pipette (trypsin digested) sample mixture through the C18 ZipTip (using 5 μ L of 0.1% TFA)
- Wash Ziptip with 0.1% TFA (2 X 10 μ L)
- Elute proteins/peptides using 2 μ L of 70% acetonitrile, on to the target plate.

RESULTS

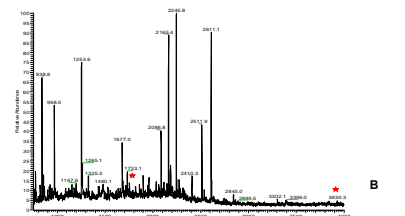
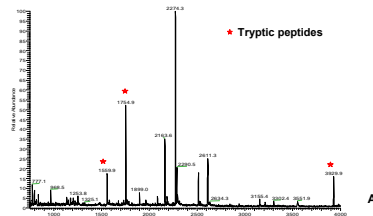


Figure 1. MS2 bacteriophage capsid protein cleaned after tryptic digestion A) by on-probe clean up and B) by ZipTip method.

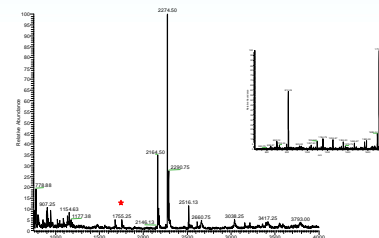


Figure 2. AP-MALDI mass spectrum and MS/MS spectrum (inset) of MS2 bacteriophage capsid protein after on-probe clean up. Concentration of MS2 was 2.5×10^6 pfu/sample (5 times lower than the limit of detection obtained using ZipTip clean up method).

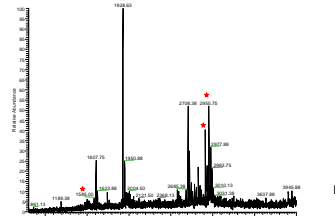
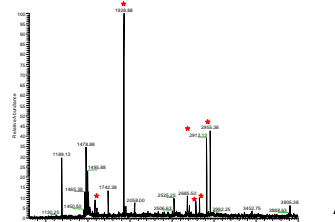


Figure 3. trypsin digested peptides from small acid soluble protein of *B. globigii* spores A) after on-probe clean up and B) without any clean up.

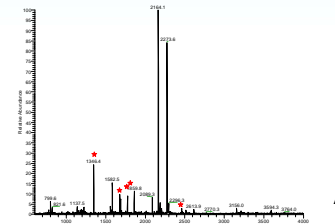


Figure 4. mass spectrum of ovalbumin in PBS after trypsin digestion A) on-probe clean up before digestion, and B) without any clean up.

CONCLUSIONS

- On Probe Clean up using C-18 hydrophobic targets
- is rapid, taking less than 10 seconds vs. 2 – 3 minutes required for ZipTip clean up.
- is more efficient, allowing for an improved sensitivity.
- Does not require additional consumables and the C-18 hydrophobic plates are re-usable.

REFERENCES

- Brockman, A.H., B.S. Dodd, and R. Orlando, A desalting approach for MALDI-MS using on-probe hydrophobic self-assembled monolayers. *Anal Chem*, 1997, 69(22): p. 4716-20.
- Brockman, A.H., N.N. Shah, and R. Orlando, Optimization of a hydrophobic solid-phase extraction interface for matrix-assisted laser desorption/ionization. *J Mass Spectrom*, 1998, 33(11): p. 1141-7.