ABRF 2005

High Sensitivity Peptide Analysis Using a Novel MALDI Substrate

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Overview

- A patterned self-assembled monolayer (SAM) that confers zones of differing wettability was constructed on a gold-coated silicon wafer to serve as a sample platform for MALDI TOF analysis.
- The usage of this substrate greatly increased the sensitivity of peptide analysis, resulting in 10 to 100-fold improvements compared to traditional MALDI TOF MS on a stainless steel plate.

Introduction

The wide application of MALDI TOF MS in proteomics has become a driving force to improve the technology towards higher sensitivity and better reproducibility. Among many other factors, sample preparation is recognized as being a critical step in the overall workflow for high sensitivity analysis. In this poster, we present our approach to improve the sensitivity of MALDI TOF MS analysis via a novel MALDI substrate.

The substrate consists of a silicon wafer with a patterned self-assembled monolayer (SAM) surface that confers zones of differing wettability (see **Figure 1**). The molecularly-flat surface allowed for concentration and precise placement of samples for MALDI mass spectrometry. The variation of wettability permits a large volume of droplet to be applied. As the sample dries, it is focused into a relatively small analysis zone, thus dramatically improving the sensitivity of analysis. Evaluations of the substrate for reproducible, high sensitivity MALDI MS of peptides and protein digests are presented.

Experimental

1. Substrate

The substrate was manufactured and provided by LumiCyte Inc. (Fremont, CA)

2. Sample Preparation Procedure

– Prepared sample solutions in aqueous 25% acetonitrile, 0.1% TFA (v/v)
– Deposited up to 5.0 µL of sample (**Figure 2**) and waited for sample to dry completely
– Added 1µL matrix solution, CHCA (Waters) 0.07 mg/ml in solvent mixture ACN:EtOH:0.1%TFA(aq), 80:10:10 (v/v/v)
– Waited for matrix to dry and analyzed by MALDI TOF MS (Waters Micromass® MALDI micro MX™)

Schematic Illustrations of the Substrate & Workflow in Sample Preparation

Figure 1. Sample well composed of differing wettability zones on the substrate surface. Order of wettability: Analysis Zone> Liquid Retention Zone > Boundary Zone

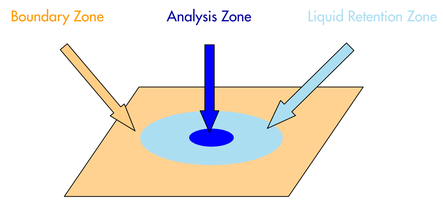
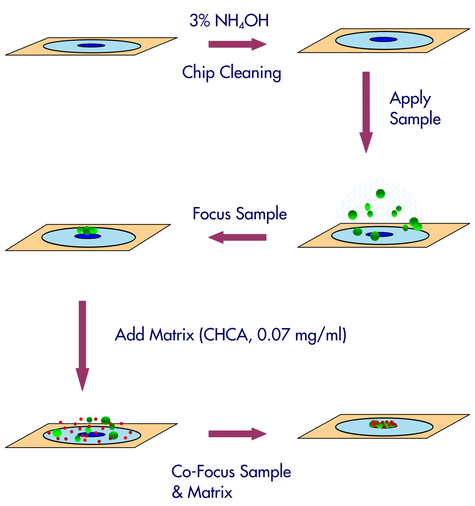


Figure 2. Sample Preparation Workflow using the Substrate



Results

Figure 3. MALDI MS spectra of a peptide standard, [Glu[1]]-Fibrinopeptide, deposited on a high sensitivity SAM-based substrate exhibiting 0.01 fmol (10 amol) sensitivity. In a typical analysis, this sensitivity is achieved at least 10 of 12 wells.

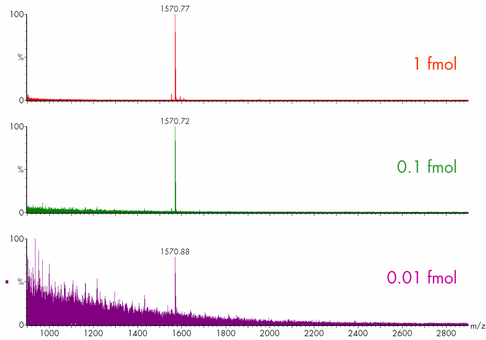


Figure 4. MALDI MS spectra of a BSA tryptic digest deposited on a high sensitivity SAM-based substrate exhibiting 50 amol sensitivity. Spectra were subjected to peptide mass fingerprinting for positive protein ID.

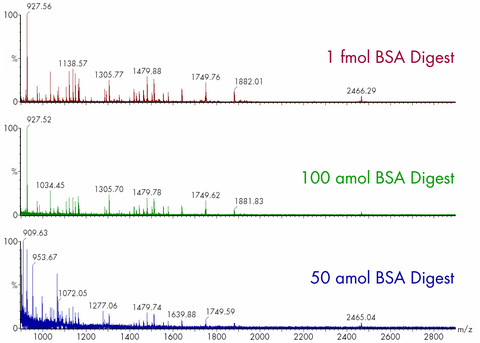


Figure 5. Comparison of the sensitivity of a SAM-based substrate and a stainless steel target for analysis of diluted in-gel tryptic digests. In-gel tryptic digests from a SDS-PAGE band (1 pmol protein loaded) were recovered with 25 µL of 25% acetonitrile. The digest extract was diluted sequentially with 25% acetonitrile/0.1% TFA solution at 10-, 100-, 1000-fold ratios. Only 1 µL of each solution was applied to each target well.

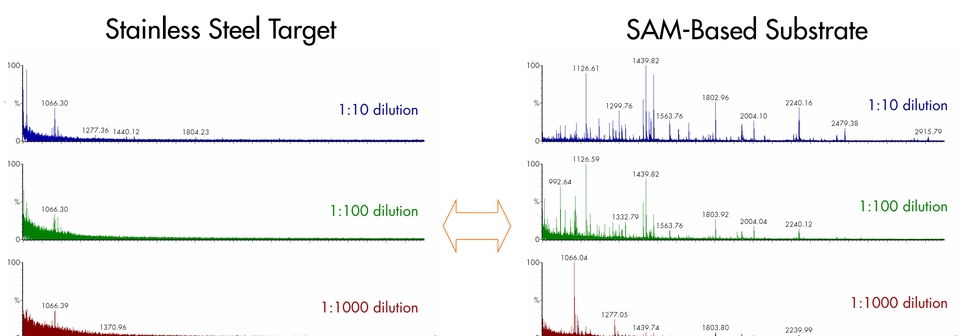
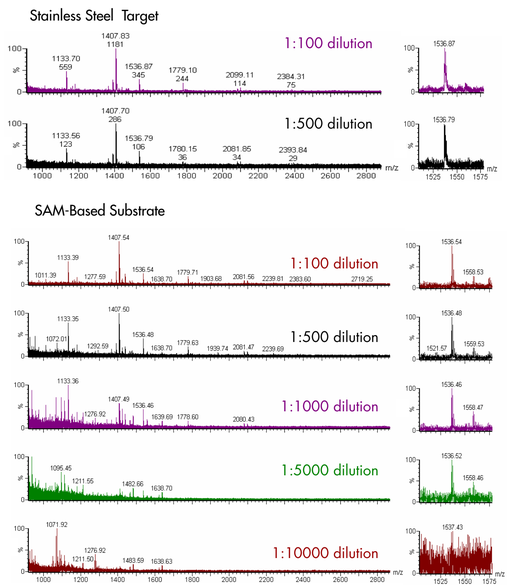


Figure 6. Comparison of target plate sensitivity for the analysis of a blind protein digest sample, with a closer view of a selected peptide. Data collection was in automated mode.



Conclusions

- A novel SAM-based MALDI substrate has been successfully constructed on a silicon wafer for high sensitivity peptide analysis.
- A sample preparation method has been developed for the usage of the target.
- An automated sample collection method has been developed for reproducible high sensitivity data collection on the target.
- The SAM-based substrate yields 10–100 fold improvements in sensitivity compared to a conventional MALDI stainless steel target.