Selective Identification of Proteins by Laser Desorption Ionization Mass Spectrometry on Photonic Nanodevices Coupled to Abiotic Receptors

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ABSTRACT

Matrix Assisted Laser Desorption Ionization Mass Spectroscopy (MALDI MS) is a particularly efficient tool for proteomics research and diagnostics with protein biomarkers. Recent innovations in the application of inorganic matrices have enabled a reduction in noise and concomitant increases in sensitivity. However, these inorganic matrices suggest even powerful new tools may be possible. Presented here are new classes of photonic nanodevice and device arrays for biomarker screening by laser desorption ionization mass spectrometry. Integration of molecular recognition and analysis elements into the highly sensitive MALDI technique for biomarker assay and discovery, as well as extension to other chemistries including small molecules, biopolymers and nanomaterials, and the incorporation of these effects into more complex device design and integration strategies.

Keywords: maldi, proteomics, nanodevices, diagnostics, biomarker

1 INTRODUCTION

Matrix Assisted Laser Desorption Ionization Mass Spectroscopy (MALDI) has been a mainstay for proteomics research and diagnostics with protein and other biomarkers. In this method, a chemical (the 'matrix') is added to the biomarker-containing sample that converts applied radiant energy (i.e., a laser) into chemical energy with sample ionization and subsequent detection by mass spectroscopy [1]. Chemical matrices as a class tend to vary from sample to sample in applicability [2] and react alone and/or with the sample during the experiment, increasing the noise in the experiment [3].

Inorganic matrices are a recent innovation for the reduction of matrix-induced noise, particularly <~3k Daltons. A large variety of inorganic nanomaterials have been reported as MALDI matrices (i.e., Surface Assisted LDI MS or SALDI) each giving an idiosyncratic spectrum of response. Here nanodots, nano-wires and even roughened surfaces and imbedded structures harvest and convert radiation into the chemical energy of ionization. These materials are largely inert to the analytes and invisible in the data due to very high effective molecular mass. These examples demonstrated that matrix noise may be reduced and gains in sensitivity could be achieved. Essentially at the same time, a new technique called Surface Enhanced LDI MS or SELDI was also developed. This MALDI-variant uses millimeter-scale areas of controlled surface chemistry to introduce selectivity to the MALDI experiment. [4] For example, a surface functionalized to be basic in character would interact more strongly with acidic proteins, and retain them after a washing step. Arrays of different surface functionality exposed to an analyte mixture, washed and then treated with matrix and analyzed give differential data sets which can be interpreted computationally to reveal the subtle differences revealing biomarkers. [5]

However, developments in templated nano materials for molecular recognition [6], separations science [7] and nano-textured materials for crystallography [8] suggested to us that the size and shape parameters of the nanostructure might also lend a new, powerful approach to selectivity to the Laser Desorption Ionization Mass Spectroscopy (LDI MS) experiment. We hypothesized that by controlling biomarker availability and alignment within inorganic nano-structures selective arrays could be achieved, improving sensitivity and reproducibility of MALDI biomarker screening. By integrating artificial receptor technology for specificity with nanostructure-based MALDI matrices for sensitivity, this technology leapfrogs ahead of previous methods (i.e. SELDI, SALDI, NIMS). The semiconductor nanofabrication techniques used to make the arrays ensure high precision and reproducibility in manufacture and performance. This hypothesis was validated through the fabrication and testing of several prototype devices to demonstrate proof-of-concept. [9]

2 EXPERIMENTAL

Silicon wafers were patterned with nanostructure arrays printed by lithographic techniques and the patterns were transferred in to silicon with fluorinated plasma reactive ion etching. The wafers were diced manually with a diamond scribe to create identical chips containing array sets. Chips for arrays 0, 1 and 2 were 20 x 10 millimeters in size and chips for arrays 3, 4, 5, 6, 7 and 8 were 5 x 10 millimeters in size. The chips were then mounted in a chip holder, which for this set of experiments merely served to support

the devices. Chips bonded with epoxy adhesive and also with no adhesive (direct chip contact with substrate) were tested.

Protein samples were prepared as 1:10 dilutions of the protein standard (dissolved in 60% CH3CN/H2O containing 0.001% trifluoroacetic acid) in CH3CN/H2O saturated with α-cyanohydroxycinnaminic acid (HCCA). The sample used for Arrays 0, 1, 2, 3, and 4 was a new vial of Protein Calibration Standard 1 (Bruker Daltonics part #206355), and the sample used for Arrays 5, 6, 7 and 8 was a new vial of Protein Calibration Standard 2 (Bruker Daltonics part #207234).

The diluted protein samples were applied to the chip surfaces by drop-casting films from 4 microliters of the 1:10 dilutions. Controls for the two experiments were prepared as an analogous spot prepared on the native Al surface. Tests without matrix contained α-cyanohydroxycinnaminic acid. The chips were then allowed to dry in air and loaded into a Bruker Daltonics Auto Flex II (Arrays 1, 2, and 3-6) equipped with a 337nm N2 laser or a Bruker Daltonics Ultra Flex III (Arrays 0, 7 and 8) equipped with a 248nm ArF laser. Laser power was adjusted to minimum giving consistent response for the control and positive ions from 20 laser pulses were collected for each spectrum.

3 PROTOTYPE RESULTS

Our prototype devices were fabricated into a system of chips mounted in a chip holder. The prototypes were then probed to determine the performance of the nanostructure arrays relative to the Al control spectrum for a sample mixture with HCCA matrix.

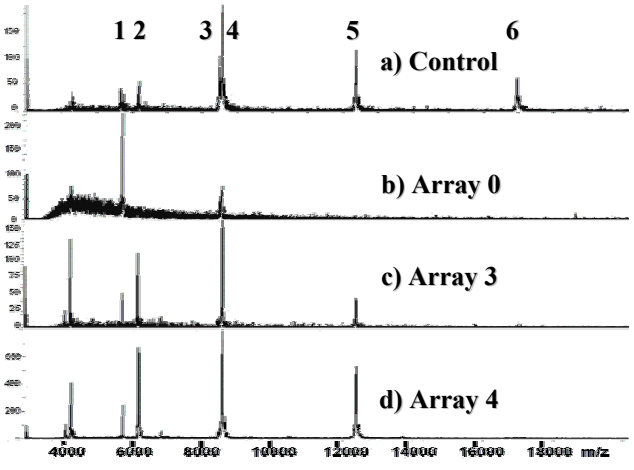

Figure 1. Results of Arrays 0, 3, 4 and aluminum control.

For one prototype (**Fig. 1**), the sample mixture on the Aluminum control (**a**) shows well resolved peaks for Insulin, **1**, M+H+ (m/z 5734.5), Cytochrome C, **2**, M+2H2+ (m/z 6181.0), Myoglobin, **3**, M+2H2+ (m/z 8476.7), Ubiquitin, **4**, M+H+ (m/z 8565.8), Cytochrome C, **5**, M+H+ (12361.0), and Myoglobin, **6**, M+H+ (m/z 16952.3).

Array 0 (**b**) shows clear selection for Insulin and elimination of ***all*** competing signals except for a trace of the Ubiquitin M+H+ signal (this was the strongest signal in the control by a factore of more than 2)..

Array 5 (**c**) shows the complete elimination of both myoglobin signals. Especially notable is the complete elimination of the Myoglobin M+2H2+ signal that strongly interferes with the Ubiquitin M+H+ signal. It is also notable that the strength of the Cytochrome C M+H+ signal is reduced in intensity, relative to the other peaks. However all of the remaining signals, including the Cytochrome C M+H+ signal are significantly stronger than the control, with the exception of both Myoglobin signal.

Array 6 (**d**) gave a qualitatively similar response to Array5. In this case, selectivity improved giving sharpened contrast between the eliminated myoglobin and retained Cytochrome C M+H+ signals. As seen in Array 5, Array 6 filtered the signal for Myoglobin from the signal for Ubiquitin. However it was also observed that array 6 gave a significantly stronger signal than Array 5 and the control, by approximately 400%.

The trend from Array 0 was seen in earlier experiments with arrays 1 and 2 (**Fig. 2**). For both Arrays 1 and 2, the signal strengths of the peaks associated with Myoglobin and Cytochrome C were substantially reduced relative to those of the control. In the case of Array 2, these peaks were completely eliminated. However only, a minimal impact from these two arrays was noted on the signal for Ubiquitin. Also notable was an overall decrease in signal strength across the sample, not seen in Array 0.

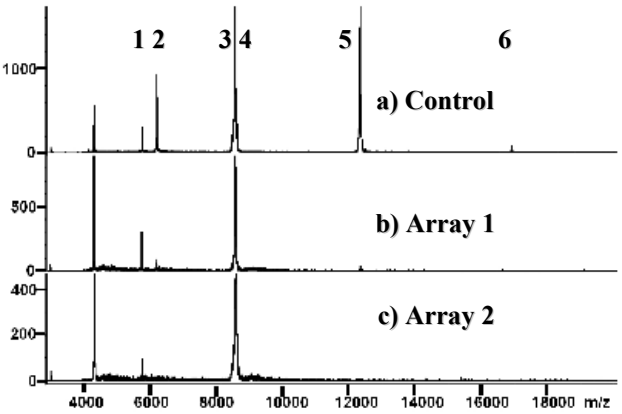

Figure 2. Results of Arrays 1, 2 and aluminum control.

The Array 1 was tested without matrix to probe for phonic performance of the nanostructures themselves

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(Fig.3). While overall intensities were reduced relative to the positive control (protein with HCCA on Al), the signal for Ubiquitin was easily resolved, and performance mirrored that of Array 1 in the presence of matrix. It is notable that the negative control (protein on Al with no HCCA) gave no discernable performance. Analogous experiments with Array 0 have not been performed. Array 2 gave poor response.

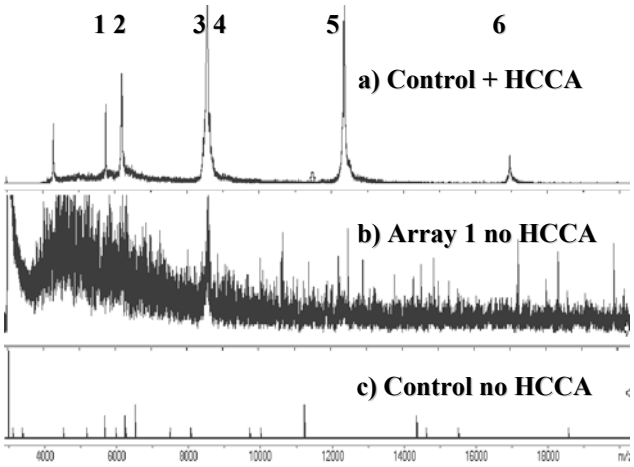

Figure 3. Performance of Array 1 with no matrix

Additional prototype testing was conducted with larger proteins (**Fig. 4**). The sample mixture on the Aluminum control (**a**) shows well resolved peaks for Protein A, **1**, M+2H2+ (m/z 22307), Trypsinogen, **2**, M+H+ (m/z 23982), Bovine Albumin, **3**, M+2H2+ (m/z 3300), and Protein A, **4**, M+H+ (m/z 44613). Below 22000 Daltons, impurities ascribed to degradation products were noted Arrows indicate signals changing across arrays 5-8.

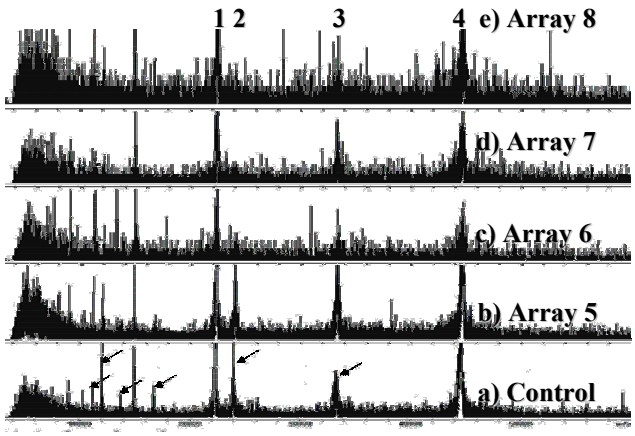

Figure 4. Performance of Array 5-8 with larger proteins

Array 5 gave a spectrum for the test proteins similar to the control, with changes noted in the detected impurity profile below 22000 Daltons. Array 6 eliminated the signal for Trypsinogen and gave a decreased response to Albumin with changes to the detected impurity profile below 22000 Daltons. Array 7 clearly selected for and eliminated the Trypsinogen signal while retaining the signals for the other proteins with again changes in the detected impurity profile below 22000 Daltons. Array 8 behaved analogously to Array 6, essentially eliminating the signals for both Trypsinogen and Albumin, again with unique perturbations in the detected impurity profile below 22000 Daltons.

4 DISCUSSION

Across the prototypes studied to date, two basic functions have been noted. Arrays may be selective ***for*** protein targets, eliminating competing signals and/or amplifying the target signal, or may be selective ***against*** targets, reducing or eliminating target signals relative to the remainder of the spectrum.

The best example for the first effect was noted for the ***selectivity of Array 0 for the diabetes biomarker Insulin***. This effect may be modulated as seen in moderated response for Arrays 1 and 2. This is interpreted as improved alignment for energy transfer and ionization in the array for the target to the exclusion of the other proteins. The second effect, best seen in ***selectivity for the heart disease biomarker Myoglobin and cancer biomarker Trypsinogen in, respectively, Array 4 and Array 7***, is interpreted as a very good fit of the protein for the nanostructure, limiting its availability when ionized to detection. This effect may also be modulated as observed in Array 3 and in Arrays 6 and 8.

These effects are believed to be analogous to other examples of artificial receptors, where binding sites are templated in separations media, membranes and other materials. However ***this is the first known report of an protein selective artificial receptor coupled to mass spectrometry***. These abiotic receptors coupled to photonic nanodevices achieve MALDI results previously noted only for affinity methods employing biomolecules recognition elements. The applicability of this method to selective detection of other biomolecules, small molecules, polymers and nanomaterials is currently under investigation.

5 SUMMARY

The hypothesis that inorganic nanostructures may show selectivity for particular protein biomarkers based on their size and shape characteristics has been upheld with the initial data collected from our first prototype devices. In this study we have shown two complimentary modalities of selection and have shown how these have been used to analyze Insulin (well known for its role in diabetes), Myoglobin, a well known biomarker for heart disease and Trypsinogen, a biomarker for some cancers. [10]

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