

## Evaluation and Application of a Microfluidics Device for Gel Sample Preparation and Analysis by AP- and OA-MALDI

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Correct preparation of gel-derived proteolytic digests for mass spectrometry is crucial for the success of subsequent analyses. Stains, salts, detergents and other excipients must be efficiently removed, and the sample sufficiently concentrated in order to obtain good results, particularly if the sample is to be directly analyzed by MALDI. Desalting and concentration of gel-derived proteolytic digests of typically 10 - 50  $\mu$ l is frequently accomplished using individual micro-packed gel-loader tips, or the commercial variant known as ZipTips<sup>TM</sup>. The micro column devices have proven very useful, but are laborious and finicky to prepare and use reliably. The latter are convenient and can be readily used with automated liquid handling robots, but offer additional challenges in their variable quality and inability to process <5  $\mu$ l.

Alternative methods have recently become available for small volume sample purification that employs microfabricated channels etched in silicon or PVDF that contain a separation media. The very low dead volumes and small internal surface area of these devices make them more ideal for desalting and concentration of gel-derived samples into final volumes of 1  $\mu$ l or less. In the present study, we have applied one such device, a disposable plastic compact disc (Gyrolab SP1) consisting of 96-integrated sample separation devices and MALDI targets, together with the Gyrolab workstation, to prepare gel derived proteolytic digests for MALDI MS using either atmospheric pressure (AP) MALDI on an Agilent 3D ion trap instrument or an orthogonal QqTof instrument from Sciex (QStarXL). The Gyrolab results are compared to those obtained using robotic cleanup using ZipTips<sup>TM</sup>.

The sensitivity, reproducibility and robustness of the Gyrolab MALDI SP1 for preparation of gel-derived proteolytic digests for MALDI analysis was tested using a standard peptide mix and digests of known loadings of gel separated proteins obtained from 1D or 2D-gels. Digests containing from 5-100 femtomole of digested protein in up to 10  $\mu$ l of digestion buffer were loaded into the receiving wells Gyrolab SP1 automatically from 96-well plates using the built-in autosampler. Up to 96 samples are simultaneously desalted, concentrated, mixed with matrix and crystallized on the integral gold-coated MALDI sample targets using centrifugal force to drive fluids through the channels and chemical coated patches or breaks to act as resist to fluid passage. The 75  $\mu$ m column on the CD is packed with a RP packing material (Source RPC). Deposition volumes are typically on the order of 200 nl onto 200 x 400  $\mu$ m spots. The resulting samples were cut into six quadrants from the CD using a cutting device supplied by Gyros. Each quadrant containing 16 samples was placed into a specially modified target plate, supplied by the vendor, that was specific to each mass spectrometer used for analysis. For ZipTip<sup>TM</sup> clean-up, samples were placed on a customized Tecan Genesis<sup>TM</sup> robotic workstation that was programmed to perform both digestions of proteins as well as sample concentration/desalting using commercially available ZipTips<sup>TM</sup>.

The standard peptide mixture consisting of 22 tryptic-like synthetic peptides in equal molar amounts was processed through the Gyrolab MALDI SP1 as well as the robotic ZipTip<sup>TM</sup> system. Identical loadings of 100, 50, 20, and 5 femtomole of the peptide mixture were processed into each segment of the disc to provide samples for comparison on the two MALDI instruments. The same amounts of the mixture were spotted onto the "calibration" areas of the disc. In this case the peptides were mixed with matrix and spotted using the systems autosampler needle onto a defined location on the disc without having passed through the microstructures. This allowed us to directly analyze identical samples from the disc either processed or not processed. Good quality mass spectra were obtained on both the Agilent AP-MALDI and the Sciex QstarXL for the 22 peptide mix, although more peptides were routinely identified from the QStarXL (20/22 @ 50fmol) as compared to the AP-MALDI (15/22 @50fmol). On these same samples it

was also possible to perform MS/MS experiments from the QStarXL. Obtaining MS/MS data from the AP-MALDI system proved to be more difficult. The very thin sample film and very small sample area resulted in rapid sample depletion under normal analysis conditions. The longest time we could collect data on this instrument was about 1.5 minutes/spot. About 3 minutes/peptide is required for good MS/MS data on the AP-MALDI. Because of this limitation the remaining experiments were carried out on the QStarXL. (Further comparisons of these two instruments can be seen on Poster WPX461). Overall quality of the data from ZipTip™ versus Gyros clean-up was similar on the QStarXL in the concentration range tested.

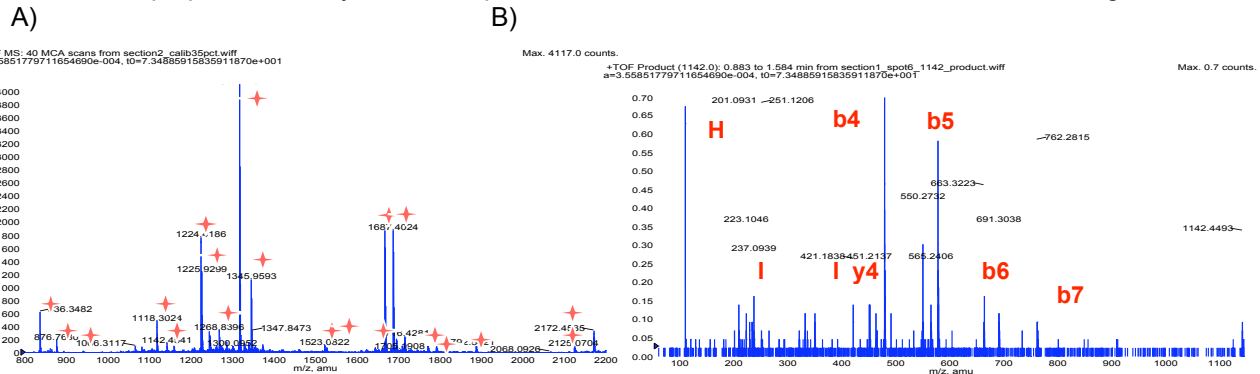


Figure 1 A) 22 Peptide mix processed through the Gyros SP1. 50 fmol of the mix loaded onto structures and analyzed using Sciex QStarXL. Masses of peptide sin the mix marked by. ✨ B) MS/MS data from peptide parent mass 1142 m/z.

For comparing in-gel digests of faintly Coomassie stained (250 fmol loaded) gel bands, BSA and myoglobin were separated by standard PAGE, the bands were excised, reduced, alkylated, and digested manually. The digests were extracted into 30  $\mu$ l and split into three 10  $\mu$ l fractions. One fraction was processed over the Gyros MALDI SP1 disc for analysis and a second fraction was processed using the robotic workstation using ZipTips™. From the 250 fmol gel band (84 fmol loaded onto either the ZipTip or into the Gyros microstructure) we were able to obtain both MS and MS/MS data on multiple peptides from single target areas.

### Conclusions:

Significantly higher ion counts and better signal to noise ratio were obtained by processing protein digests over the Gyrolab SP1 disc as compared to automated ZipTip™ clean-up. Both approaches gave data of similar quality on two different MALDI instruments. (Sciex QStarXL and Agilent AP-MALDI).

Another advantage that the Gyrolab workstation has over ZipTips™ lies in individual reproducibility of spot formation. A matrix spot was obtained from each microstructure in every experiment with the Gyrolab SP1 disc; whereas in our hands, the variability between the ZipTips™ resulted in a properly formed matrix spot not being obtained about 10% of the time.

Due to the smaller spot size and thin film, only a single MS/MS spectra can readily be obtained from a single Gyros spot. This will require the use of multiple spots from the same sample to acquire multiple MS/MS data.