A new MALDI target with 1536 spots per plate is described. The new AnchorChip™ target provides substantial improvements to the LC-MALDI analysis of complex biological samples. The MTP AnchorChip 1536 TF can accommodate fractions collected from an extended LC gradient (up to four hours) or, alternatively, from multiple shorter LC gradients spotted in sequence. Complete MALDI-TOF/TOF analysis of all the spotted fractions can be easily achieved within a single run without the need of target exchange. This provides significant improvements regarding utilization of the high throughput capacity offered by high speed MALDI-TOF/TOF systems such as Bruker’s ultrafleXtreme™. Applying expanded LC gradients to the LC-MALDI analysis of complex proteomics samples leads to an improved depth of analysis resulting in a higher number of protein IDs and peptide assignments. In the benchmark example shown here, a single injection of 500 ng E.coli cell lysate digested with trypsin yielded more than 900 protein IDs based on more than 6000 assigned peptides.

**Introduction**

High speed MALDI-TOF/TOF instruments, for example Bruker’s ultrafleXtreme [1], are perfectly suited for the in-depth LC-MALDI analysis of highly complex proteome samples. Being equipped with latest technology, i.e. smartbeam™ II laser [2], PAN ion focusing and FlashDetector™, these instruments allow highly complex peptide mixtures to be processed at 1 kHz acquisition speed in MS and MS/MS mode and at an increased level of resolution and mass accuracy. [3] To fully explore the benefits offered by high speed MALDI-TOF/TOF instruments, target plates are required that can accommodate large spot numbers. Such high capacity targets allow the use of longer gradients in LC-MALDI analyses, which enables better separation of complex samples and, thus, achieves improved depth of analysis by more comprehensive MS/MS analysis. Furthermore, these targets take full advantage of the instrument’s capacity for high throughput by providing huge sample numbers for sequential analysis without the need of target exchange over long periods of time, i.e. overnight or over an entire weekend.

In this technical note we introduce the MTP AnchorChip 1536 TF target, a new type of plate that provides four times more spot positions compared to conventional MTP 384 targets. The new target is used here for the LC-MALDI analysis of 500 ng E.coli cell lysate trypsin digest with an LC gradient of more than 3 hours, which yielded 1152 fractions. The benefits provided by the new target format are a substantial reduction of overall analysis time, increased instrument efficiency due to its extended cycle of continuous operation, and a significant increase in the number of protein identifications achieved.
Experimental

Sample:
An amount of 500ng of E.coli trypsin digest was injected on column.

LC-MALDI-TOF/TOF method parameters:
The following LC-MALDI methodology was used:
NanoLC system: Bruker EASY-nanoLC™ II
Trap column: Nanoseparations RP-18 capillary, 100 µm x 2 cm, 5 µm particles
Analytical column: Dionex Pepmap 100, 75 µm x 15 cm, 3 µm particles
Column flow rate: 300 nl/min
Eluent A: 0.05% TFA in water
Eluent B: 0.05% TFA in a mixture containing 90% ACN and 10% water
Gradient: 2 … 45% B within 192 minutes

The eluate was spotted onto an MTP AnchorChip 1536 TF target plate using a Bruker PROTEINEER fc™ II fraction collector. LC fractions, automatically mixed with α-cyano-4-hydroxycinnamic acid MALDI matrix (HCCA) on the fly, were deposited on the target every 10 s, starting 10 min after the analytical gradient had started. LC system and fraction collector were operated under control of Bruker’s Hystar™ software (version 3.2 SR2, including LC-MALDI AddOn 1.4).

MALDI-MS and -MS/MS analysis was performed on a Bruker ultraflexXtreme operating under the control of Compass™ for Flex software 1.3 (FlexControl™ 3.3, FlexAnalysis™ 3.3). 4000 laser shots were accumulated per spectrum in both MS and MS/MS mode. MS spectra were externally calibrated using Bruker Peptide Calibration Standard II. Non-redundant selection of precursor peptides for MS/MS was performed by the WARP-LC™ 1.2 software applying a signal to noise threshold of 7.

Results

Fig. 1 shows an MTP AnchorChip 1536 TF target spotted with 1152 fractions from a 192 min nanoLC gradient utilized in the LC-MALDI analysis of 500 ng E.coli trypsin digest. The figure illustrates the extremely precise spot alignment achieved by the pre-structured AnchorChip surface [4,5]. This enables 100 % spot finding efficiency of the laser while rastering across the well-defined sample spot area. The MTP AnchorChip 1536 TF target is equipped with a transponder. This allows the target geometry to be correctly recognized by all software modules involved. For example, the Hystar software, upon fraction collection on the PROTEINEER fc II, will automatically omit those spots on the plate which are intended for use as external calibrant positions (see Fig. 1). Each calibrant spot is surrounded by eight sample spots, enabling highly precise next-neighbour calibration for all target positions.

Fig. 2 shows example LC-MALDI run sequences set up for a readily prepared MTP 1536 target. The new high capacity AnchorChip plate can accommodate fractions from long LC gradients, as shown in Fig. 2a) for a 192 min gradient. Alternatively, multiple shorter LC runs, for example from 2DLC, GeLC or label-free LC-MALDI proteomics experiments, can be spotted in series on the same plate (see Fig. 2b) and are then processed as a batch. In any case, the high number of spots deposited on the plate ensures...
efficient utilization of the instrument capacity without the need of target exchange, even when running the MALDI-TOF/TOF at kHz speed over a weekend. The outstanding crystalization homogeneity that is achieved on AnchorChip plates results in high quality MS and MS/MS data. At the same time, these matrix preparation spots are extremely robust against laser irradiation and thus allow large numbers of MS/MS spectra to be acquired from individual target spots. Fig. 3, as an example, illustrates this for the LC fraction spot collected at retention time 104.33 min, where 31 precursors were selected for MS/MS. The fragment ion spectra displayed in the bottom part of Fig. 3 were obtained from precursors ranked in the acquisition order at position 1, 20 and 29 for this individual spot, i.e. were acquired when the spot had already seen 80,000 and 116,000 laser shots (4000 shots accumulated per spectrum) respectively. The resulting MS/MS data, however, was of high quality to allow unambiguous identification of \textit{E.coli} peptides, which clearly illustrates the outstanding spot capacity offered by the new MTP 1536 AnchorChip target.

The new 1536 format MALDI target also provides significant improvements with respect to the protein identification rate. Use of a 192 min LC gradient with 1152 fractions spotted onto a single MTP 1536 AnchorChip plate resulted in 939 identified proteins based on more than 6000 assigned

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**Figure 2:** Setup of autoXecute runs for readily prepared targets is done by a few mouseclicks using the respective wizard in the WARP-LC run editor. a): The new MTP AnchorChip 1536 TF target can accommodate fractions from an LC gradient up to 4 hours long. b): Alternatively, multiple shorter gradients can be spotted in series, and are then batch processed with the MALDI instrument.

**Figure 3:** SurveyView displayed in ProteinScape resulting from LC-MALDI analysis of 500 ng \textit{E.coli} cell lysate trypsin digest. In the MS spectrum recorded from the fraction spot at $t_r = 104.33$ min, 31 precursors were selected for MS/MS. Displayed fragment ion spectra correspond to precursors ranked in the acquisition order at position 1, 20 and 29 for this particular spot, and, i.e. were acquired after the spot had already seen up to 116,000 laser shots. However, all three MS/MS spectra led to unambiguous identification of peptides originating from \textit{E.coli} proteins illustrating the outstanding spot capacity of the new 1536 format AnchorChip target.
peptides. In Fig. 4, these results are compared in detail with the results obtained from an earlier LC-MALDI approach [3] that involved a shorter gradient (128 min) spotted over two MTP384 formatted target plates (in total 768 fractions). Approximately 20% more proteins are identified when using the new 1536 format target in conjunction with an expanded LC gradient, reflecting an increased depth of analysis. At the same time, the overall analysis time required for completing the LC-MALDI experiment was dramatically shortened due to the fact that the MALDI acquisition was done within a single overnight run, without the need of target exchange.

**Summary**

The new MTP AnchorChip 1536 TF target, offering space for up to 1536 spots per plate, was described here. The increased spot count facilitates the LC-MALDI analysis of highly complex proteomic samples that require separation by reversed phase LC gradients up to 4 hours long. Alternatively, fractions from multiple shorter LC gradients can be spotted in series, which is of special benefit to 2DLC, GeLC and label-free proteomics experiments. In any of these workflows, the new MTP AnchorChip 1536 TF enables the user to fully benefit from the high throughput capacity offered by high speed MALDI-TOF/TOF instruments.

Using the new target in conjunction with an extended 192 min LC gradient, 939 proteins were identified by LC-MALDI analysis of 500ng *E. coli* trypsin digest. This represents a 20 % increase compared to the result obtained from a previous LC-MALDI method relying on a shorter gradient spotted over two 384 formatted targets. Furthermore, the analysis time was shortened to a single overnight run as the new 1536 format target eliminated the need of plate exchange during the LC-MALDI experiment.

**References**

[3] Bruker Application Note MT-100: Accelerated nanoLC-MALDI-TOF/TOF analysis of complex proteomics samples performed at a new level of resolution and mass accuracy

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- proteomics
- protein identification
- LC-MALDI
- speed
- high throughput
- MALDI preparation

**Instrumentation & Software**

- EASY nanoLC II
- PROTEINEER fc II
- ultrafleXtreme
- ProteinScape

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