

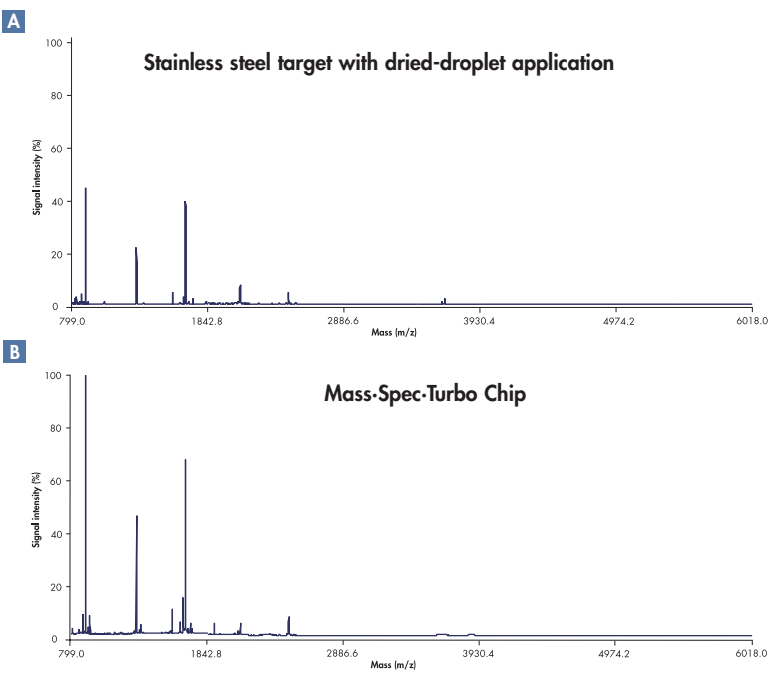
Sensitive and reproducible detection of peptides by Maldi-TOF MS using self-assembled monolayer (SAM)-based chips – specifications and applications



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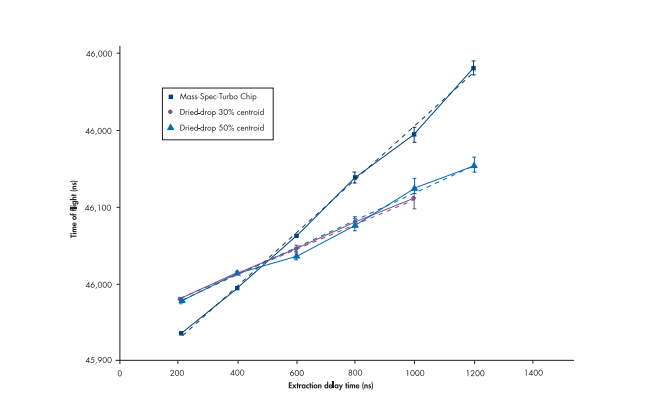
Introduction



A mixture of the peptides des-Arg¹ Bradykinin, Angiotensin I, Glu1-Fibrinopeptide B (50–100 fmol each) diluted in 20% ACN/0.1% TFA were prepared either by **A** standard dried-droplet preparation with CHCA on a stainless steel plate or **B** by application on a Mass-Spec-Turbo Chip, onto which the same matrix was pre-deposited by sublimation prior to sample loading. Note the higher sensitivity using the Mass-Spec-Turbo Chip as demonstrated by an increased S/N ratio and peak height of the respective peptides.

Sensitivity, mass accuracy, and reproducibility are prerequisites for efficient MALDI-MS analyses used in current quantitative proteomic work-flows. Our approach consists of a novel technology for peptide sample preparation using plates with pre-deposited matrix spots of extra-fine sub-micron-size CHCA crystals prepared by vacuum sublimation on an ultraphobic surface (Mass-Spec-Turbo Chips). Such a design facilitates a highly reproducible signal detection of peptides in the low attomole range providing a gain in performance in MALDI-TOF MS compared to standard sample preparations. Besides the beneficial effect of the ultraphobic surface of the chip, we investigated whether matrix sublimation is another crucial feature enabling the higher sensitivity we observed. Here we present the results of our experiments on the effect of the CHCA matrix crystal structure on the initial velocity of the peptide ions and signal intensities. Furthermore, we compared the internal energy of ions generated during the MALDI process of dried droplet preparations with preparations on sublimated CHCA (Mass-Spec-Turbo Chip) with the help of thermometer molecules.

Initial velocity of ions — Experimental methods



Flight time vs. delay time for (insulin + H)⁺ prepared on pre-sublimated CHCA matrix (Mass-Spec-Turbo Chip) or standard dried-droplet CHCA prep. .

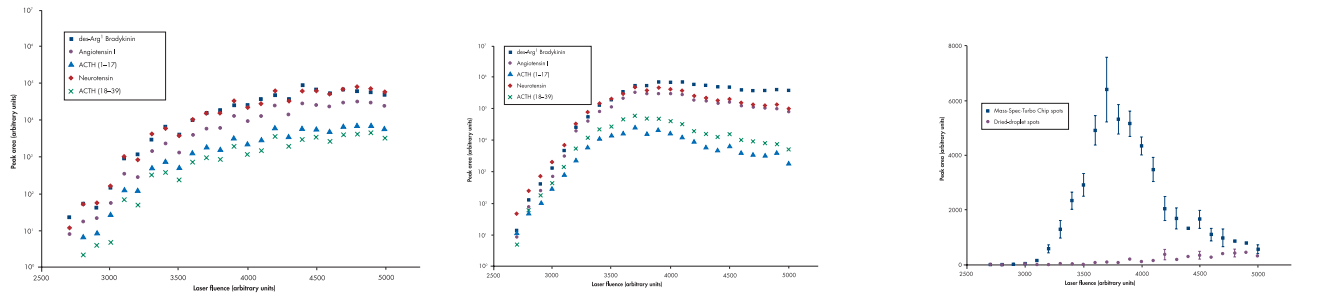
Peptide	m/z (mono)	CHCA-preparation initial ion velocity (m/s)	Mass Spec Turbo Chip initial ion velocity(m/s)
des-Arg1-Bradykinin	904.5	143±38	221±15
Angiotensin I	1296.7	181±9	289±6
Glu1-Fibrino-peptide B	1570.7	210±9	283±14
Neurotensin	1672.9	239±3	354±14
ACTH (1-17)	2093.1	194±10	n.d.
ACTH (18-39)	2465.2	213±9	402±16
ACTH (7-38)	3657.9	215±9	n.d.
Insulin (bovine)	5730.6	250±3	522

■ On Mass-Spec-Turbo Chips, the initial velocity of peptides is significantly higher than standard dried-drop preparations.

Table shows initial velocities of different peptides prepared using standard dried-droplet technique or Mass-Spec-Turbo Chips. Values were calculated with peak detection settings at 50% centroid. Average values of 6 to 8 independent recordings summarizing approx. 30 shots each are shown.

Laser fluence and signal intensity

In the previous experiments we demonstrated the surprisingly different behaviour of analytes prepared on sublimated CHCA matrix in terms of initial ion velocity and stability of molecules (internal energy) in the MALDI process. Since signal yield (and therefore sensitivity) is associated with analyte stability, we carried out comprehensive studies on the dependence of signal intensity on laser fluence. Using the same laser fluence, signal intensity is increased by at least one order of magnitude on Mass Spec Turbo Chips compared to classical dried droplet (DD) preparations. In contrast to DD preparations, the peak does not get saturated upon increasing laser fluence, but seems to peak in a certain range. **Therefore, when using Mass-Spec-Turbo Chips, laser fluence must be carefully adjusted and optimized to achieve maximum sensitivity and performance.**

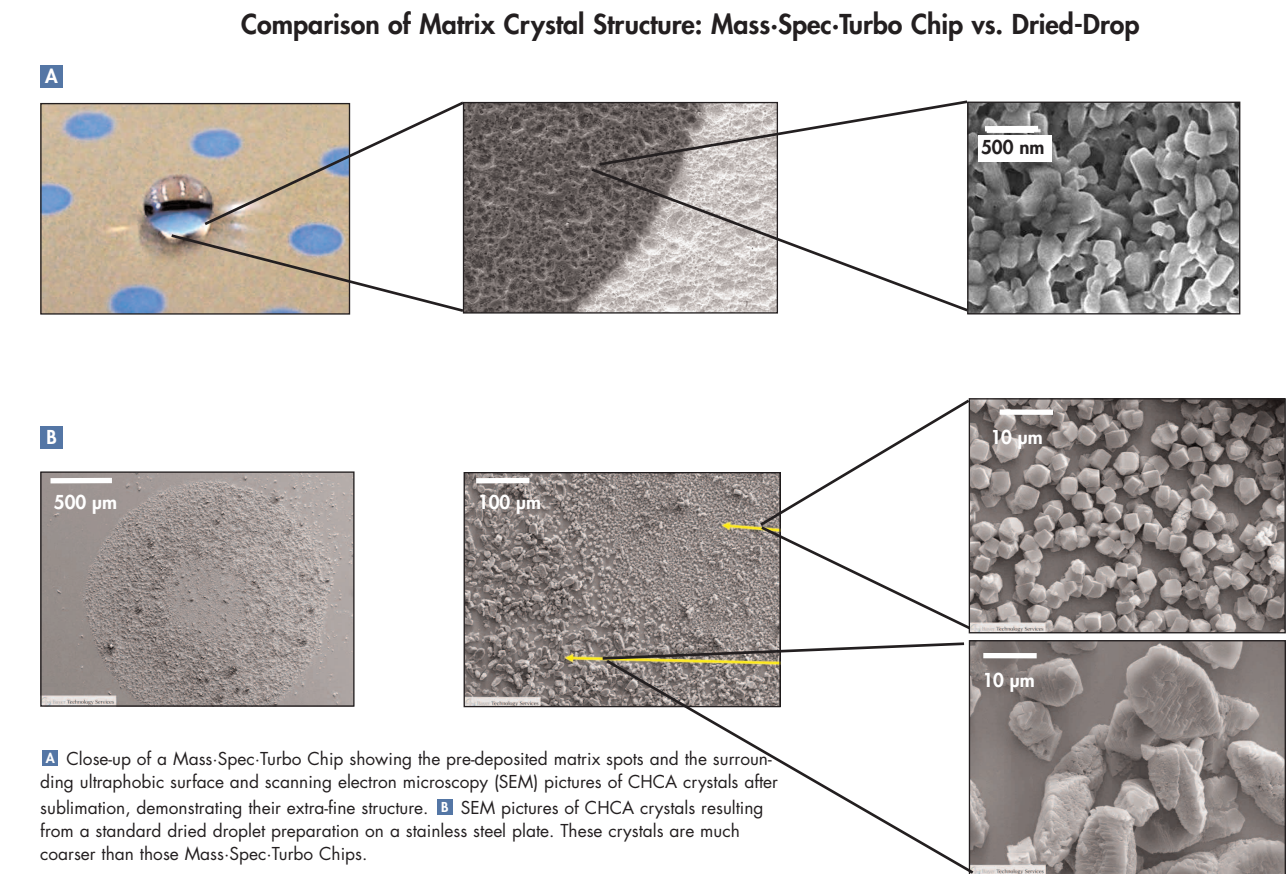


Peak areas of different peptides (50–450 fmol each) as a function of laser fluence. A peptide mixture was applied as standard CHCA dried droplet preparation on stainless steel target. Note: Peak area is presented on logarithmic scale.

Peak areas of different peptides (50–450 fmol each) as a function of laser fluence. Peptide mixture was applied directly on sublimated CHCA matrix (Mass-Spec-Turbo Chips). Note: Peak area is presented on logarithmic scale.

Comparison of ACTH (18–39) peak areas (150 fmol) prepared as a dried-droplet preparation or on a Mass-Spec-Turbo Chip as a function of laser fluence. Peak areas are depicted on a linear scale.

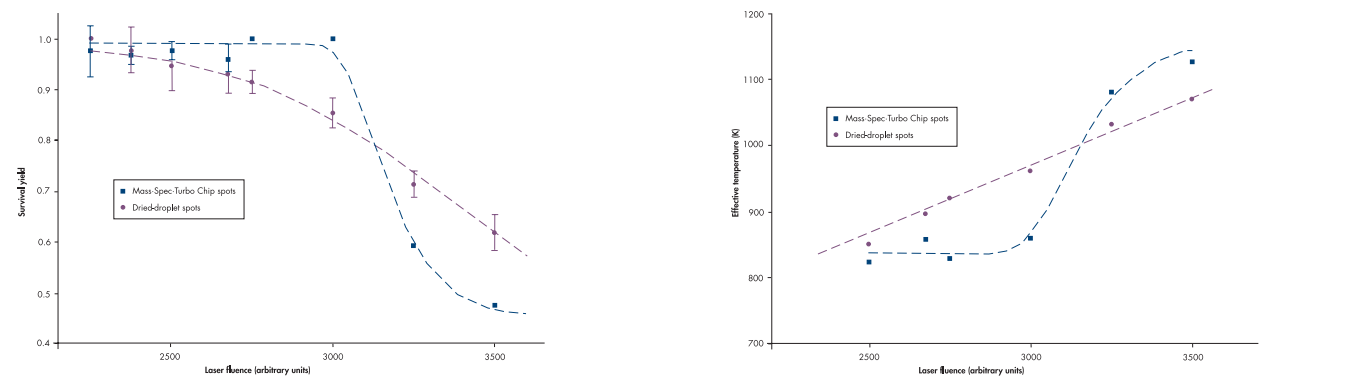
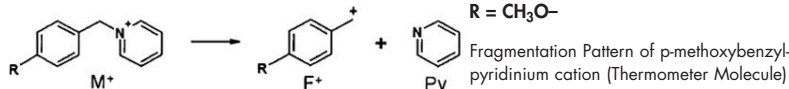
Ultra-fine homogeneous matrix structure



A Close-up of a Mass-Spec-Turbo Chip showing the pre-deposited matrix spots and the surrounding ultraphobic surface and scanning electron microscopy (SEM) pictures of CHCA crystals after sublimation, demonstrating their extra-fine structure. **B** SEM pictures of CHCA crystals resulting from a standard dried droplet preparation on a stainless steel plate. These crystals are much coarser than those Mass-Spec-Turbo Chips.

Internal energy and thermometer molecules

To investigate, whether an increased initial ion velocity is associated with a lower internal energy = higher ion stability a technology published by Luo et al. (3) using so called thermometer molecules (TM) were applied. The rational behind this methodology lies with the simple fragmentation pattern combined with well-studied reaction dynamics of the fragmentation process allowing calculation of the internal energy. The survival yield (SY) indicative for the stability of the molecule is defined by: $SY = I(M^+) / (I(F^+) + I(M^+))$



Survival Yield (SY) of p-methoxybenzylpyridinium cation (TM) after preparation on Mass-Spec-Turbo Chips or as standard dried-droplet (DD) preparation as a function of laser fluence. A higher SY indicates an increased stability of the intact TM during the first 100 ns of the MALDI process. Each data point represents the average value for 6 replicates of 2, 8, 32, and 128 pmol sample spots.

Comparison of the effective temperature [K] of TM prepared on sublimated matrix spots (Mass-Spec-Turbo Chips) or as standard DD preparation as a function of laser fluence. Each data point represents the average value for 6 replicates of 2, 8, 32, and 128 pmol sample spots.

Conclusions

- Mass-Spec-Turbo Chips provide a higher sensitivity for the analysis of peptides in MALDI-TOF MS compared to standard dried droplet preparations.
- The CHCA matrix sublimated on ultraphobic sample plates shows a significantly smaller crystal size and possesses remarkably different features compared to common dried droplet preparations using the same compound.
- The initial velocity of the ions is significantly higher. Consequently, for optimal performance the matrix.txt file in the instrument software should be changed. Instructions are available at www.qiagen.com
- The internal energy of a thermometer molecule formed during the MALDI process is decreased in a specific range of laser power; thus, a higher stability of analytes can be achieved if laser fluence is adapted.
- In contrast to standard CHCA dried droplet preparations signal intensity follows an optimum-like curve upon increasing laser fluence. Consequently laser fluence must be carefully adjusted to achieve highest sensitivity.
- We believe the higher sensitivity seen in these experiments is due to the concentration of the analyte in the top matrix layers in combination with an increased stability = decrease of PSD effects on the molecules within a defined laser range. Future work will focus on the fragment yields of peptides prepared on Mass-Spec-Focus Chips.

References
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(2) Glückmann, M., and Karas, M. (1999) J. Mass. Spectrom. **34**, 467.
(3) Luo, G., Marginean I., and Vertes A. (2002), Anal. Chem. **75**, 6185.
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