

## Developing a timsTOF fleX method for enhanced sensitivity in higher $m/z$ MALDI-MS measurements

A method was developed that optimizes the sensitivity of the timsTOF fleX instrument in high  $m/z$  MALDI-MS mode.

### Abstract

Utilization of adjusted MS tuning parameter settings and adjusting the “TIMS In” gas pressure to ~1.8 mbar results in significantly increased signal intensities for MALDI ions in the high  $m/z$  range up to  $m/z$  20,000 and beyond. The optimized instrument setup enhances intact mass analysis of proteins, oligonucleotides and other biologics taking advantage of MALDI speed and throughput in combination with high resolution and mass accuracy provided by orthogonal TOF-MS.

Keywords: timsTOF fleX, MALDI, high  $m/z$ , proteins, oligonucleotides, biologics, biopharma, applied

### Introduction

The Bruker timsTOF fleX instrument features an ESI/MALDI dual ion source for seamless switching between ionization modalities within seconds providing access to an expanded application space.

Matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) offers unparalleled speed of analysis making it a highly attractive method for routine application workflows. This is especially significant for larger cohorts of samples, for example quality control (QC) analyses in biopharma, bioengineering and further branches of industry.

However, as MALDI in general yields molecular ions of low charge states, best possible instrument sensitivity in the high  $m/z$  range is an important precondition for the successful detection of larger sized molecules by MALDI-MS.

In this technical note we describe how to set up the timsTOF fleX for high  $m/z$  MALDI-MS measurements. The high-mass setup uses adjusted MS tuning parameters together with a reduced "TIMS In" gas pressure of approximately 1.8 mbar resulting in improved detection sensitivity for large  $m/z$  ions ranging up to  $m/z$  20,000 and beyond. This dedicated instrument setup should enhance the routine analysis of larger sized molecules such as intact proteins, oligonucleotides and other biologics at high MALDI speed and throughput.

## Experimental

### Materials

The following standard compounds were purchased from Merck (Germany): Myoglobin from horse heart (product number M1882); carbonic anhydrase isoenzyme II from bovine erythrocytes (product number C2522); phosphorus (red) (product number 04004).

MALDI matrices  $\alpha$ -cyano-4-hydroxycinnamic acid (HCCA, product number 8201344) and 2,5-dihydroxyacetophenone (2,5-DHAP, product number 8231829) as well as ESI-L low concentration tuning mix (product number 8245631) were from Bruker (Billerica, MA, USA).

### Sample preparation

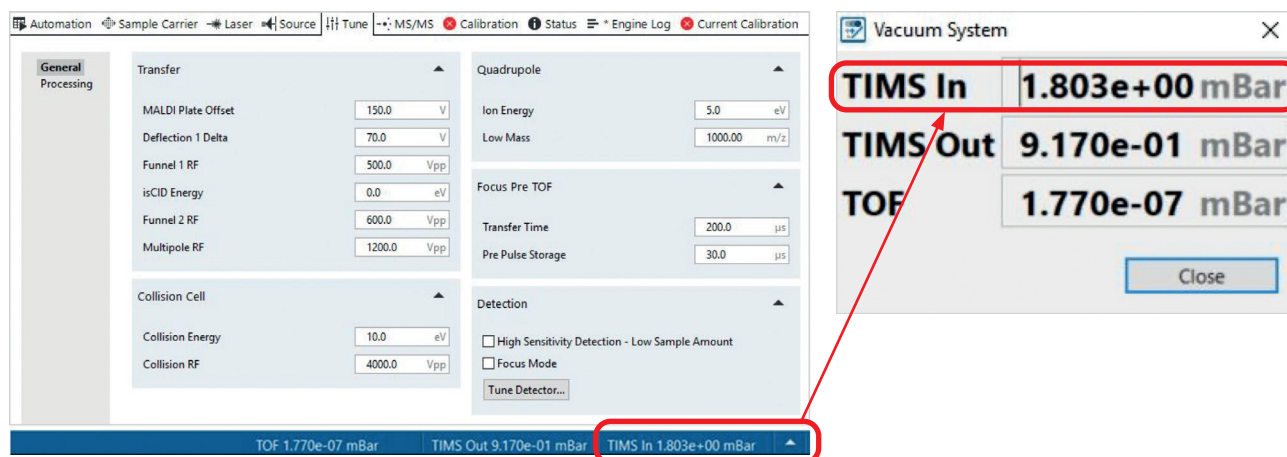
All samples were prepared on a Bruker MTP Anchorchip 384 BC target plate according to default protocols described in the instructions-for-use document provided with the target plate. HCCA and 2,5-DHAP, respectively, were used as MALDI matrices.

Crushed red phosphorus was suspended in acetone by means of sonication. 0.5  $\mu$ L aliquots of the resulting suspension were spotted on the MALDI plate without addition of a MALDI matrix.

### Mass spectrometry

MALDI-MS measurements were performed on Bruker timsTOF fleX instruments in positive ion polarity mode with Trapped Ion Mobility Spectrometry (TIMS) turned off. The timsControl acquisition method in use was tuned for optimum ion transmission in the high  $m/z$  range 8000–20,000. Optimized MS tuning parameters are reported in Figure 1.  $m/z$  calibration was performed by means of matrix-free laser desorption ionization (LDI) of red phosphorus as a reference substance.

Direct infusion ESI measurements of low concentration tune mix were performed at a supply flow rate of 180  $\mu$ L/h operating the instrument in positive ion TIMS on mode.



**Figure 1**

Screenshot taken from the timsControl software GUI (left) showing MS tuning parameter settings applied in high  $m/z$  MALDI-MS measurements.

The red frame on the blue ribbon in the bottom indicates the "TIMS In" gas pressure display. For better visualization of the current pressure setting, a magnified display (right) can be activated by clicking the arrow icon in the bottom right corner of the blue ribbon.

Adjustment of the “TIMS In” gas pressure (i.e. the pressure in the MALDI ion source region) was performed by turning the black wheel accommodated on the instrument’s front side, right above the syringe pump. The current “TIMS In” pressure value can be read from the respective display on the blue ribbon in the bottom right corner of the timsControl GUI (see Figure 1). For better real-time visualization during pressure adjustment, a magnified pressure display can be activated by clicking the respective arrow icon shown on the blue ribbon in the bottom right corner of the timsControl GUI (see Figure 1).

#### Data processing:

Recorded mass spectra were processed in Bruker DataAnalysis software using the following parameter settings:

##### Isotopically resolved spectra:

Smoothing: Savitzky Golay, auto  $m/z$  width, 1 cycle  
 Baseline correction: Flatness 0.8  
 Peakfinder: SNAP (version 2)  
 Quality factor threshold 0.3

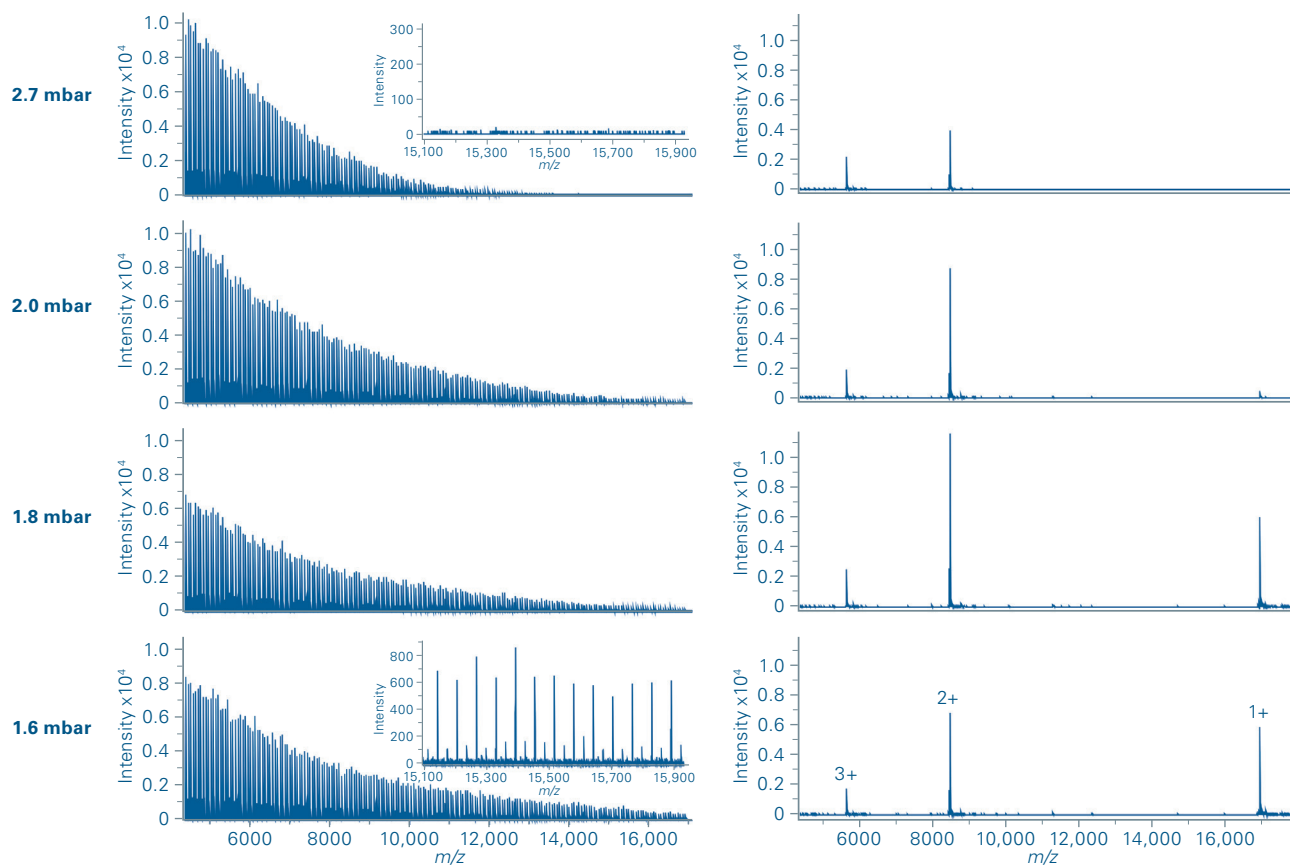
##### Average mass spectra (protein A):

Smoothing: Savitzky Golay, 2.8  $m/z$  width, 1 cycle  
 Baseline correction: Flatness 0.8

## Results

### MS tuning parameters for enhanced $m/z$ sensitivity

To achieve optimum ion transmission in the high  $m/z$  range, timsControl MS method parameters were adjusted accordingly. Optimized MS tuning parameter settings are given in Figure 1. The



**Figure 2**

LDI-MS spectra (4000 laser shots accumulated per spectrum) of red phosphorus (**left**) and MALDI-MS spectra of myoglobin (16 pmol on spot, 2,5-DHAP matrix, 2000 laser shots accumulated per spectrum) (**right**) acquired under varying “TIMS In” pressure conditions.

option "High Sensitivity Detection - Low Sample Amount" should be activated only in case of weak signal because of low sample amount. To ensure optimum mass accuracy, the  $m/z$  calibration should be updated whenever High-Sensitivity Detection has been activated/deactivated.

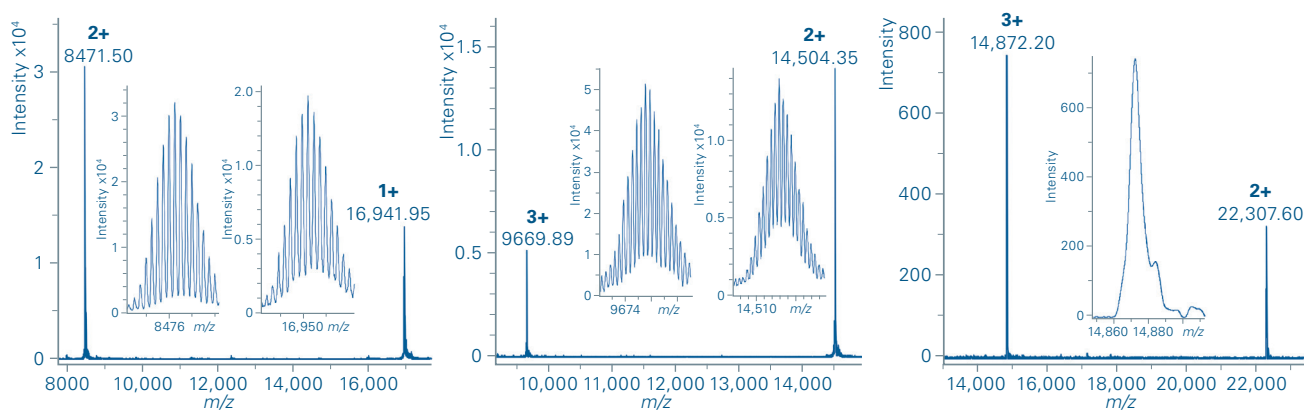
### Adjusting the "TIMS In" gas pressure

High-mass sensitivity is enhanced significantly by adjusting the "TIMS In" pressure to a value of approximately 1.8 mbar. For individual instrument units, the optimum pressure setting should be determined by experiment using suitable reference standards, for example red phosphorus and intact myoglobin (horse). Red phosphorus, upon LDI (no MALDI matrix required), forms a regular pattern of monoisotopic cluster ions across an ultrawide  $m/z$  interval ranging up to  $m/z$  20,000 and beyond. Myoglobin, a 17 kDa protein yields isotopically resolved MALDI molecular ion signals of low charge states (1+, 2+ and 3+ mainly; MALDI matrix: HCCA or 2,5-DHAP) distributed over the  $m/z$  range between  $m/z$  5500 and 17,000. Both of these reference substances are, therefore, well suited for monitoring the local sensitivity along the  $m/z$  scale under varying "TIMS In" pressure conditions. Figure 2 displays LDI-MS spectra of red phosphorus and MALDI-MS spectra of intact myoglobin, respectively, acquired at "TIMS In" gas pressure gradually reduced in the range between 2.7 and 1.6 mbar. Decreasing the pressure below 2.0 mbar yielded a significant gain in signal intensity in the high  $m/z$  range around  $m/z$  8000 and beyond. Therefore, 1.8 mbar was used as "TIMS In" pressure setting in the following high-mass MALDI-MS measurements.

### High $m/z$ MALDI-MS measurements utilizing the optimized instrument setup

Figure 3 displays example MALDI-MS spectra obtained from various intact proteins when applying the optimized high-mass instrument setup.

For proteins of a molecular weight up to 30 kDa approximately, e.g. myoglobin (17 kDa) and carbonic anhydrase isoenzyme II (29 kDa), molecular ion signals (most abundant charge states 1...3+) appear isotopically resolved yielding exact monoisotopic mass information at low to sub-ppm accuracy level. MALDI-MS spectra obtained from even larger sized proteins, e.g., recombinant protein A (44 kDa; most abundant charge states 2+, 3+), deliver reliable average mass.



**Figure 3**

**MALDI-MS spectra obtained from standard proteins when applying the optimized high-mass instrument setup:**

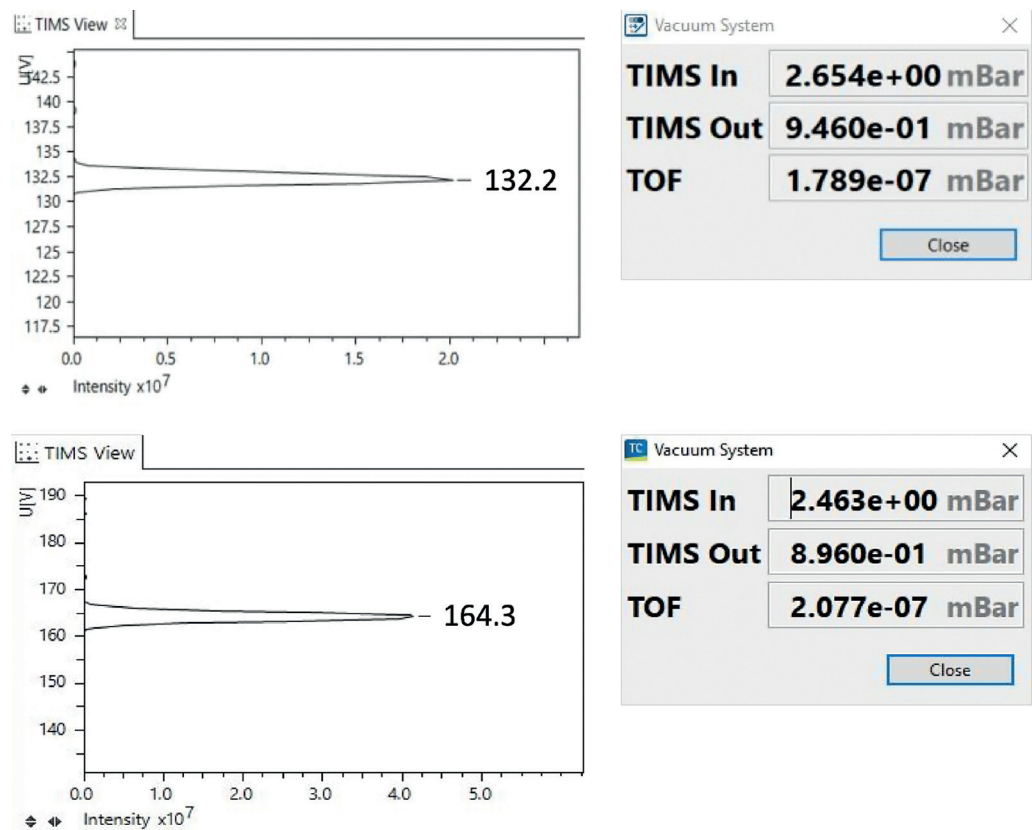
**Left:** Myoglobin (MW 17 kDa, 1.6 pmol on spot, HCCA matrix, 4000 laser shots); **Center:** Carbonic anhydrase II (MW 29 kDa, 16 pmol on spot, 2,5-DHAP matrix, ,000 laser shots); **Right:** Recombinant protein A (MW 44 kDa, 16 pmol on spot, 2,5-DHAP matrix, 35,000 laser shots)

High-mass sensitivity depends on the analyte's molecular weight and the charge state distribution of the resulting MALDI ions. Smaller sized intact proteins like myoglobin (17 kDa) can be detected down to femtomole amounts per MALDI sample spot. Proteins of larger molecular weight require 10-20 picomole per sample spot to yield MALDI mass spectra of high quality.

MALDI-MS intact mass analysis of other types of larger sized molecules, e.g. oligonucleotides, also benefits from the optimized instrument setup described above. Furthermore, the high-mass setup enhances the sequence readout achievable in MALDI top-down N- and C-terminal sequencing of intact proteins (Next-Generation MALDI-TDS) [1].

### Setting the instrument back to default pressure conditions

To get back to standard operating conditions typically used in 4D-Omics or MALDI Guided SpatialOMx® applications (proteomics, lipidomics, metabolomics etc.), the respective timsControl default application method should be loaded, and low concentration ESI tune mix should be infused via the standard ESI interface. The "TIMS In" gas pressure should be adjusted to a level yielding a stable ion mobility peak maximum for  $m/z$  622 at a trapping potential [U] of 132 V (instrument units with TIMS cartridge #1875139) and 164 V (instrument units with TIMS cartridge #1894787), respectively. Typically, this results in "TIMS In" pressure values of approximately 2.6 mbar and 2.4 mbar, respectively. The information regarding TIMS cartridge # of your instrument unit can be looked up in the "About" menu in timsControl. In a final step, recalibration of the ion mobility dimension should be performed.



**Figure 4**

Setting the "TIMS In" pressure back to its default value brings the instrument back to standard operating conditions typically used in 4D-Omics applications

**Top:** Default "TIMS In" pressure settings for instruments with TIMS cartridge #1875139; **Bottom:** Default "TIMS In" pressure settings for instruments with TIMS cartridge #1894787.

## Conclusions

- An optimized instrument setup is provided increasing the sensitivity in high-mass MALDI-MS measurements in the  $m/z$  range up to 20,000 and beyond.
- The described high-mass instrument setup comprises adjusted MS tuning parameters and utilization of reduced “TIMS In” gas pressure (1.8 mbar, approximately).
- The optimum “TIMS In” pressure setting should be determined by experiment monitoring the local sensitivity along the  $m/z$  scale under varying pressure conditions using suitable reference substances (e.g. red phosphorus and myoglobin).
- The optimized instrument setup enhances the analysis of larger sized molecules such as proteins, oligonucleotides and other biologics at MALDI speed and throughput in combination with high resolution and mass accuracy provided by orthogonal TOF-MS.

## References

- [1] Asperger A, Resemann A, Evers W, Goedecke N, Suckau D. *Next-generation MALDI top-down sequencing of protein biotherapeutics – expanding the scope of timsTOF technology*. Bruker Application Note LCMS-186. [www.bruker.com](http://www.bruker.com)

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