TIMS-MALDI MS

timsTOF MALDI PharmaPulse

Unbiased, deep high-throughput screening by label-free mass spectrometry

Innovation with Integrity

Taking label-free HTS to the next level

timsTOF MALDI PharmaPulse® (MPP) represents the ultimate solution for MS based label-free high-throughput solution (HTS) and uHTS taking full advantage of Bruker's innovative timsTOF technology.

MALDI mass spectrometry (MS) has proven itself true uHTS capability, enabling primary screens comprising 1 million compounds and more [1,2]. timsTOF MPP combines the proven speed and robustness of MALDI, a key factor for fully automated 24/7 high-throughput operation, with a unique level of assay specificity based on ultrafast gas-phase separation by Trapped Ion Mobility Spectrometry (TIMS) and accurate-mass TOF-MS detection, allowing for new, deeper insights into molecular interactions at HTS speed.

timsTOF MALDI PharmaPulse® serves as a highly capable platform for near real-time verification of chemical synthesis products keeping the pace of synthetic chemistry high-throughput experimentation (HTE) in early drug discovery, and providing access to an expanded analyte space by means of MALDI-2 laser post-ionization technology.

[1] Winter, M. et al.; SLAS Technol. 2019, 24, 209–221
[2] Simon, R. P. et al.; SLAS Discov. 2020, 25, 372–383

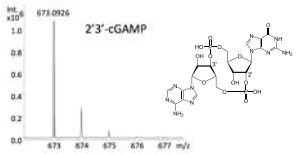
Accelerating drug discovery with MS based screening

Mass spectrometry offers huge potential for enhancing the efficiency in early drug discovery. MS enables fast, label-free, highly sensitive and specific assay read-out providing access to drug targets previously not accessible by label-based assay technologies.

Label-free
- Tremendous cost savings
- Enhanced physiological relevance
- Minimized interferences

Sensitivity and Specificity
- Lowered False Discovery Rate (FDR)
- Reduced demand for hit confirmation by orthogonal assay methods

HTS based on **Mass Spectrometry**



New target space
- Providing access to drug targets not addressable by label-based assay technologies

Multiplexing
- Monitoring multiple analytes at a time
- Reduced sample consumption
- Even higher throughput

Deeper insights into molecular interactions enabled by unique timsTOF MPP performance

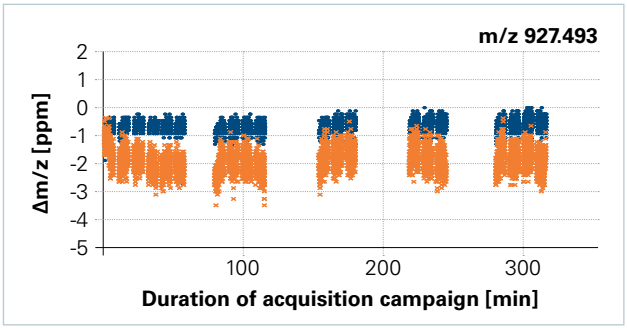
Key technology feature	HTS related benefits
Proven robustness of MALDI	• True **uHTS** capability • **Maximum** system **up-time**
Dual ESI/MALDI ion source: 10 kHz smartbeam 3D laser	• **Fast** reading rates of up to 3 wells/sec • **Ultrahigh throughput** enabling primary screens of **> 1 million** compounds
QTOF-MS: Accurate mass; isotopic fidelity; CID-MS/MS	• Enhanced assay **specificity and sensitivity** at uncompromised speed • Extended **linear range** of **quantitation** • Reduced **FDR** • Highly confident verification of **compound ID** (synthesis products)
Trapped Ion Mobility Spectrometry (TIMS)	• Additional dimension of ultrafast **separation** in the gas phase • **Faster** than SPE or LC (typical timescale ≤ 1 sec) • Reduced **background** interferences (separation of **isobars** and **isomers**) • **CCS aware** confirmation of **compound ID** (synthesis products)
MALDI-2 laser post-ionization	• **Expanded chemical space**

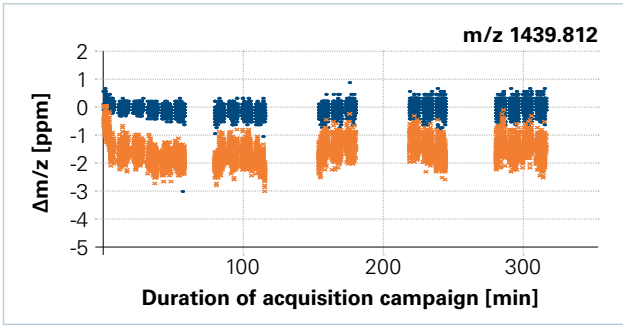
Exceptional data quality delivered at HTS speed

timsTOF MPP delivers accurate-mass data throughout large acquisition campaigns at high acquisition speed - a key requirement for success in uHTS.

Readout of a 1536 formatted sample plate in high-resolution MS or MS/MS mode takes less than 10 minutes (reading rate up to 3 wells/sec).

Mass stability, a critical indicator of system robustness, was monitored for two peptide ion signals over an acquisition time period of > 5 hours (20 x 384 = 7860 data points). Low to sub-ppm mass accuracy was maintained throughout the entire acquisition campaign ensuring continuously high quality of HTS results.





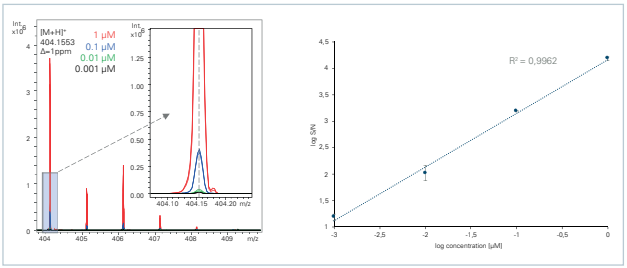
× External calibration
● Internal recalibration (Lock mass: m/z 1296.6848)

High-quality data turned into meaningful HTS results

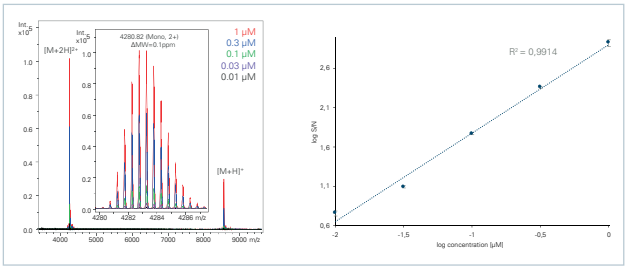
Outstanding quality of timsTOF MPP raw data enables efficient quantitative feature extraction for a broad variety of target molecules ranging from small drug-like compounds up to larger sized peptides turning raw MS data into meaningful HTS results.

Low to sub-ppm mass accuracy ensures high level of convidence in target signal assignment **avoiding false-positive hits**.

Titration curve obtained for perphenazine (MW 403 Da) over 3 orders of magnitude concentration range (0.001 – 1 µM)

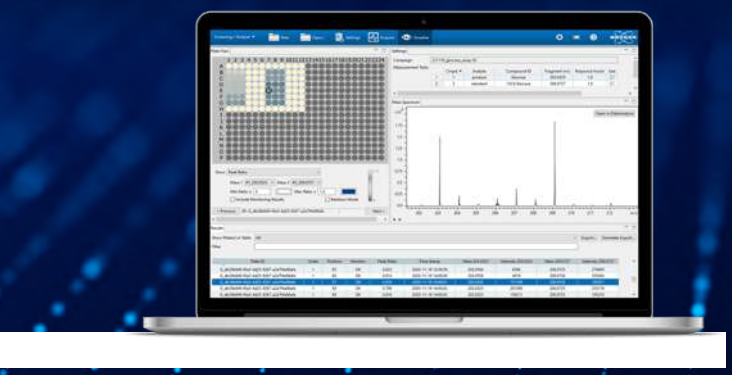


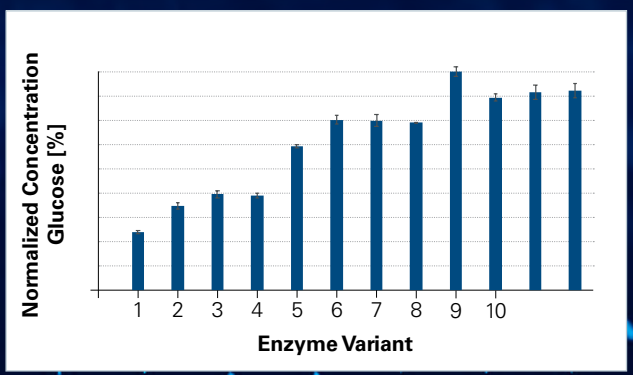
Titration curve obtained for ubiquitin (MW 8558 Da) over 2 orders of magnitude concentration range (0.01 – 1 µM)



Enzyme activity screening enabled at shortest time to result

Label-free monitoring of enzyme activity is delivered by timsTOF MALDI PharmaPulse at HTS speed and highest level of performance.





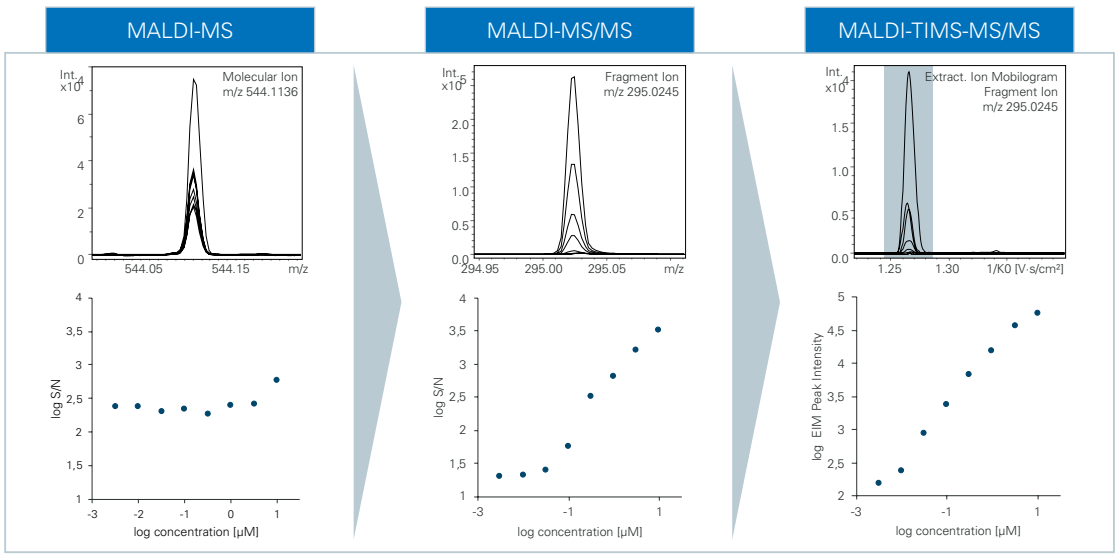
Sample courtesy: Prof. Peter Westh, Department of Biotechnology and Biomedicine, Danmarks Tekniske Universitet, Lyngby, Denmark

In a pilot study, differently bioengineered enzyme variants were assayed for their activity by quantifying glucose as a product of enzymatic conversion, using $^{13}C_6$ glucose as an internal standard.

High-resolution accurate-mass data delivered by timsTOF MPP allowed for robust and reliable target feature extraction resulting in low RSD values between 0.1 and 3.7%.

Assay specificity enhanced by innovative timsTOF technology

Advanced timsTOF MPP operation modes efficiently resolve background interferences, enhancing quantitation results



Sample courtesy: Dr. Frank H. Büttner & Team, Drug Discovery Sciences, Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany

Quantitation of a target molecule (MW 543.1 Da) in MALDI-MS mode disrupted due to overlap with assay background.

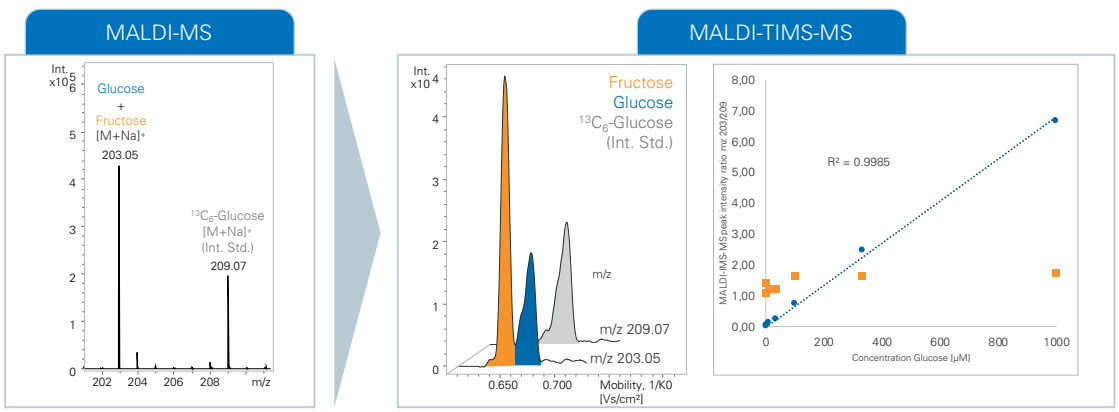
Analysis of target-specific fragment ion m/z 295.0 in MALDI-MS/MS mode yields enhanced quantitation results.

TIMS separation upfront to MS/MS enables virtually interference-free quantitation of the target molecule.

The TIMS benefit: Adding another dimension to HTS for fast separation of isobars and isomers

TIMS is capable of separating isobars and isomers on a timescale significantly faster than LC or SPE (typically ≤ 1 sec per separation cycle). TIMS, therefore, enables interference-free quantitation of target compounds indistinguishable by mass spectrometry alone.

Example: Quantitative MALDI-TIMS-MS analysis of glucose ($C_6H_{12}O_6$) in presence of isomeric fructose ($C_6H_{12}O_6$)



Accelerating chemical synthesis screening

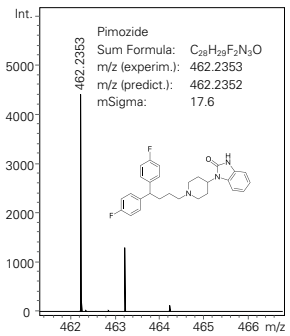
Keeping the pace of HTE chemistry in early drug discovery

In search of new drug molecules, high-throughput experimentation (HTE) creates vast libraries of newly designed compounds through multi-variant high-throughput chemistry. A key bottleneck in this process is the rapid analysis and confirmation of chemical reaction products.

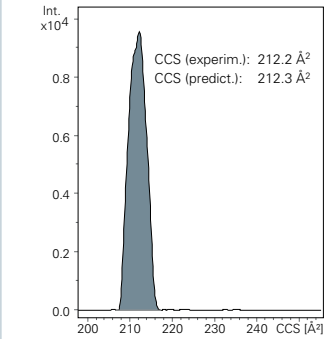
timsTOF MPP enables near real-time verification of chemical synthesis products by measuring multiple physical properties, i.e. accurate molecular mass, isotopic pattern, collisional cross-section (CCS) and, on-demand, CID-MS/MS fragmentation data, reducing HTE feedback time and chemical costs *[3]*.

[3] Bruker Application Note LCMS-182; www.bruker.com

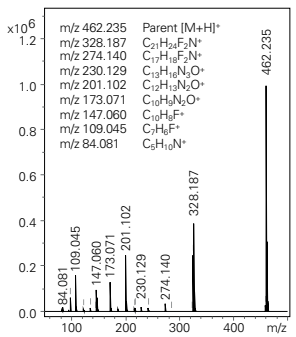
UHR-TOF MS: Accurate Mass + Isotope Pattern



TIMS: Collisional Cross Section (CCS)



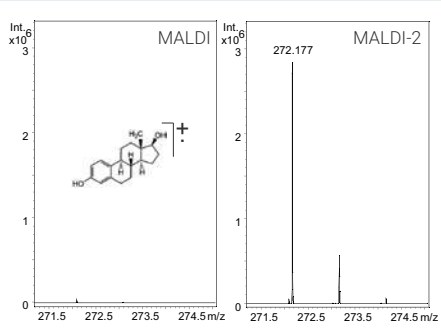
CID-MS/MS: Structure-Specific Fragmentation



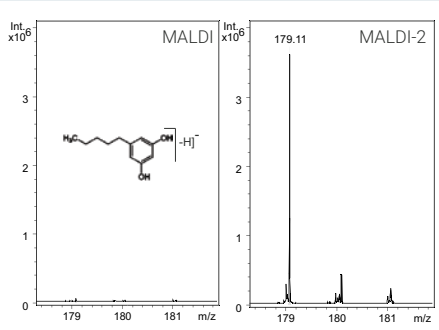
Expanded chemical space with MALDI-2 technology

MALDI-2, a groundbreaking new technology based on laser post-ionization, enhances the detectability of certain classes of compounds that used to be out of scope of conventional MALDI.

Estradiol



5-Pentylresorcinol



timsTOF MALDI PharmaPulse – a complete HTS solution

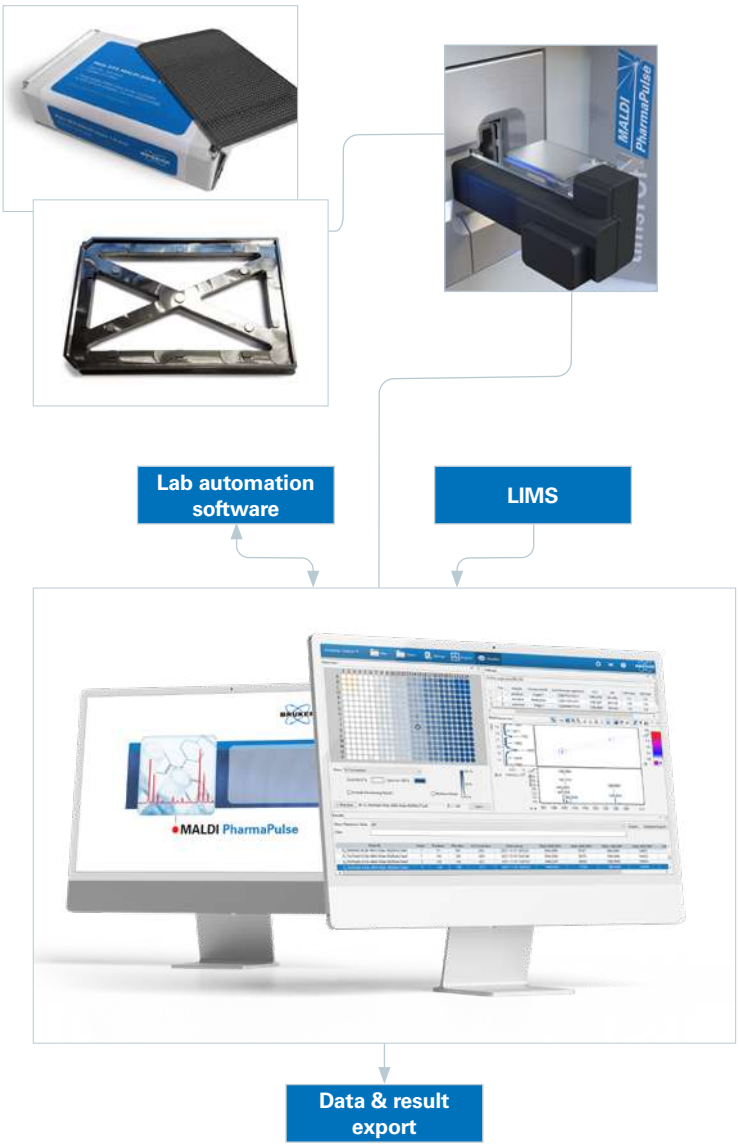
Bruker timsTOF MALDI PharmaPulse is an integrated solution comprising hardware and software components specifically developed to support HTS and uHTS workflows

Disposable MALDI sample plates for automated MALDI preparation by high-performance liquid handlers in any format ranging from 96 to 1536 and beyond.

Light-weight plate adapter specifically designed for safe handling by lab robotics.

timsTOF fleX instrument equipped with

- Dual ESI/MALDI ion source
- Autoloader for plate exchange by a robotic arm
- MALDI-2 (optionally)



MALDI PharmaPulse 2023 software

Dedicated HTS software for timsTOF MPP

- **Seamless setup and execution** of fully automated screening campaigns utilizing various timsTOF MPP operation modes (MS, MS/MS, TIMS).
- **Workflow support** for a broad range of screening and further high-throughput applications, including
  - Mechanistic assays (biochemical, cell-based)
  - Cell uptake assays
  - Binding assays (Affinity-Selection MS)
  - Phenotype screening
  - Synthesis monitoring
  - Compound library validation.
- **Automation Interface** for integration with automation scheduling software (e.g. ThermoFisher Scientific, HighRes Biosolutions, Analytik Jena).
- **Result Viewer** for instant evaluation of raw data and results.
- **Export Interface** for data and result transfer to external software (e.g. Genedata Screener).

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Further insights into molecular interactions – in real time

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