



## Application Note # MT-100

# Accelerated nanoLC-MALDI-TOF/TOF Analysis of Complex Proteomics Samples Performed at a New Level of Resolution and Mass Accuracy

Sample complexity continues to be one of the most challenging aspects for researchers in the field of expression proteomics. Various separation techniques are commonly applied at both the protein and peptide level to reduce the complexity of a proteome to be analyzed. However, despite these efforts, the number of compounds present in proteomics samples may still be considerable. Mass spectrometers are invaluable tools for the analysis of such complex proteomics samples. Their effectiveness is directly related to the ability to achieve several factors simultaneously, these include:

- fast acquisition speed
- high resolution and mass accuracy
- high sensitivity and dynamic range
- long term robustness and reliability

### Introduction

The recently introduced ultrafleXtreme™ MALDI-TOF/TOF mass spectrometer sets a new benchmark with regard to all of the above criteria. It features an impressive array of unique technological innovations (Table 1) to provide a level of performance in terms of resolution, sensitivity, mass accuracy and robustness, which has never been achieved before on any other MALDI-TOF/TOF platform. To illustrate the advances in performance provided by the

ultrafleXtreme in the analysis of highly complex proteomics samples, an *E.coli* digest sample was chosen here as an example.

### Experimental

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

#### LC-MALDI-MS/MS instrumentation

LC system	Bruker EASY-nanoLC
Trap column	Nanoseparations C18, 20x0.1mm
Analytical column	Dionex PepMapC18, 75µm i.d., 15cm length
Solvent A	0.05% TFA in water
Solvent B	0.05% TFA in 90% ACN
Gradient	2-45% B in 128min
Column flow rate	300nl/min
Fraction collection	Bruker Proteineer fc II, 768 fractions, 10s each
MALDI target	MTP Anchorchip 800-384
MALDI matrix	alpha-Cyano-4-hydroxycinnamic acid (HCCA)
Mass spectrometer	Bruker ultrafleXtreme MALDI-TOF/TOF, operated under control of Bruker's Compass 1.3 software

**Table 1: Innovative key features and related improvements in performance on the new ultrafleXtreme MALDI-TOF/TOF**

Innovative features	Resulting improvements
<b>smartbeam-II™ laser kHz system electronics</b>	acquisition at kHz speed in MS and MS/MS mode (over entire mass range and at full digitizer frequency)
<b>PAN (Panorama focussing) FlashDetector™</b>	broadband resolution up to <b>R&gt;40,000</b>
<b>FlashDetector™ 4GHz Digitizer</b>	low to sub-ppm <b>mass accuracy</b> : <b>≤5ppm</b> (external calibration) <b>≤1ppm</b> (internal calibration)
<b>Perpetual™ MALDI ion source (laser-based self cleaning)</b>	long term <b>robustness</b> <b>minimum instrument down-time</b> (short cleaning cycle time; instrument ready for next acquisition within <b>15min</b> )

Data analysis: Data analysis was performed in ProteinScape, Bruker's proteomics database system. MASCOT was used as a search engine applying the following parameters:

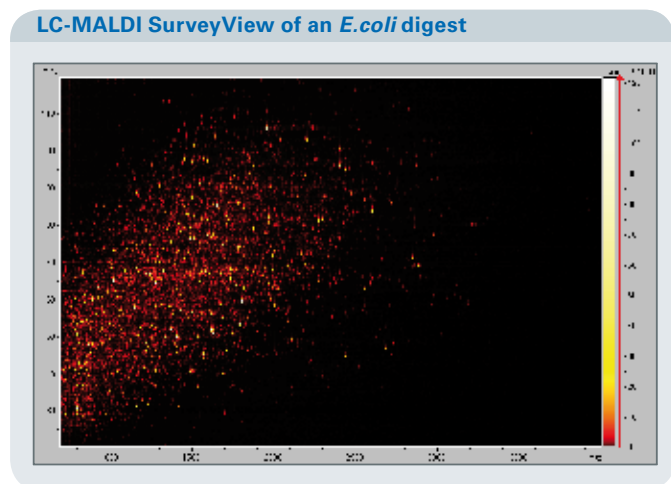
Database	Swissprot
Taxonomy	Escherichia coli
Enzyme	I) Trypsin (maximum 1 missed cleavage) II) None
Precursor mass tolerance	15ppm
Fragment mass tolerance	0.5Da
Optional modifications	Profile I (short profile)
	Carbamidomethyl (C)
	Oxidation (M)
	Profile II (extended profile)
	Carbamidomethyl (C,E,H,K)
	Oxidation (M,H,W)
	Dioxidation (W)
	Acetyl (Protein N-term)
	Deamidated (N,Q)
	Dethiomethyl (M)
	PyroGlu (N-term Q,E)

Fig. 1: SurveyView plotting retention time vs. m/z, obtained from nanoLC-MALDI-MS analysis of 500ng E.coli digest. More than 18,000 peptide compounds were detected with S/N>3.

## Results

More information generated within reduced timeframe

The ultrafleXtreme is the fastest MALDI-TOF/TOF currently available. The instrument is fully capable of acquiring data at 1kHz laser repetition rate in both MS and MS/MS mode. In the LC-MALDI analysis of 500ng *E.coli* digest described here, the time required for the generation of a single MS spectrum (2500 shots at 1kHz laser repetition rate) was approximately 3 seconds. Accordingly, acquisition of all 768 MS spectra covering the 2h LC gradient was completed within 38 minutes. MS/MS spectra were also acquired at kHz speed (3,500 shots/spectrum), yielding an averaged acquisition time per MS/MS of ≤ 5 seconds. After completion of an extensive LC-MALDI experiment, the laser-based self-cleaning device implemented in the ultrafleXtreme's new Perpetual™ MALDI source is of great advantage for rapid and efficient cleaning of the ion source, resulting in a minimized down time of the instrument of approximately 15 minutes until the next experiment can be started.



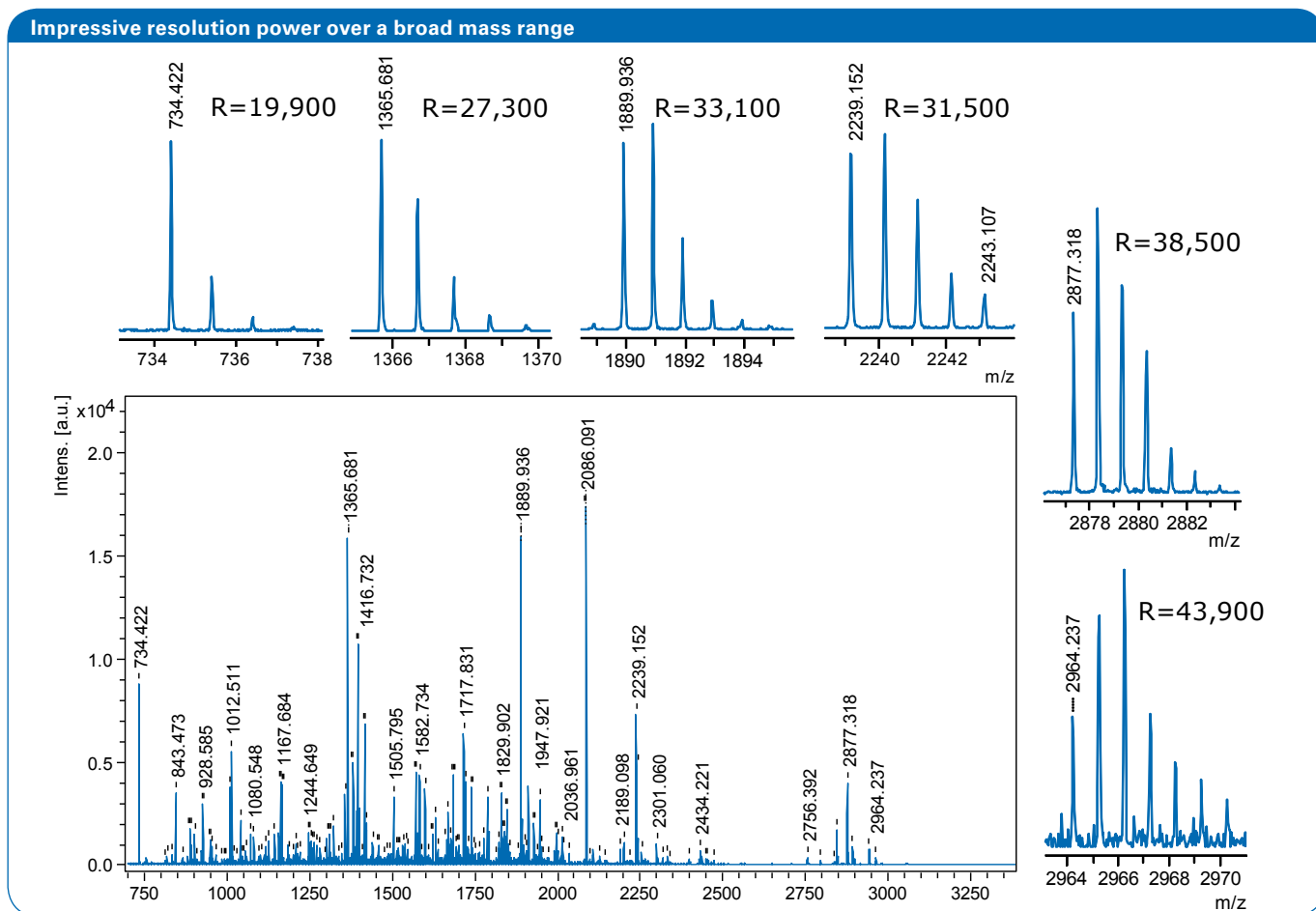


Fig. 2: MALDI-MS spectrum of a spotted LC fraction ( $t_R=69\text{min}$ ) obtained from nanoLC separation of 500ng *E.coli* digest. The ultrafleXtreme provides a new level of resolution over a broad mass range, ranging up to  $R>40,000$  for larger peptides.

### Addressing sample complexity by unparalleled resolution and mass accuracy

The ultrafleXtreme offers a level of resolution that has never been achieved on a MALDI-TOF/TOF before. Figure 1 shows the LC-MALDI-MS heat map obtained from 500ng *E.coli* digest. More than 18,000 peptides ( $S/N>3$ ) were detected during the 2 hour gradient. Figure 2 shows an example MALDI-TOF spectrum obtained from LC fraction eluting at  $t_R=69\text{min}$ . Resolution of the detected peptide peaks ranges from 20,000 to more than 40,000 spanning over a broad mass range from 700 to 4000Da. The impressive resolving power provided by the ultrafleXtreme is of great advantage to recognize partially overlapping peaks, and, thus, contributes significantly to an enhanced confidence level in precise peptide mass measurement.

To fully exploit the instrument's performance in terms of resolution and mass accuracy, the newly implemented FlashDetector™ can be operated at digitizer frequencies of up to 4 Gigasamples per second (GS/s). Figure 3 illustrates

the impact, which the digitizer rate has on achieving optimum mass accuracy. For high resolution peptide signals, an increased digitizer sampling rate of 2 GS/s results in significantly improved mass accuracy, compared to detection at 1 Gs/s, due to the increased number of data points describing the spectral peaks.

As shown above, enhanced instrument resolution together with lossless peak detection are directly related to improved mass accuracy. As a further illustration of this, the distribution of mass errors is plotted in Figure 4 for all peptides assigned to *E.coli* proteins in the LC-MALDI analysis of 500ng *E.coli* digest. An averaged mass error of 4.98ppm is achieved by external calibration taking into account thousands of peptides covering a dynamic range of multiple orders of magnitude. 90% of all peptide matches were within 10ppm, 56% within 5ppm, and 36% were detected with less than 3ppm mass deviation.

### Mass accuracy optimized by FlashDetector

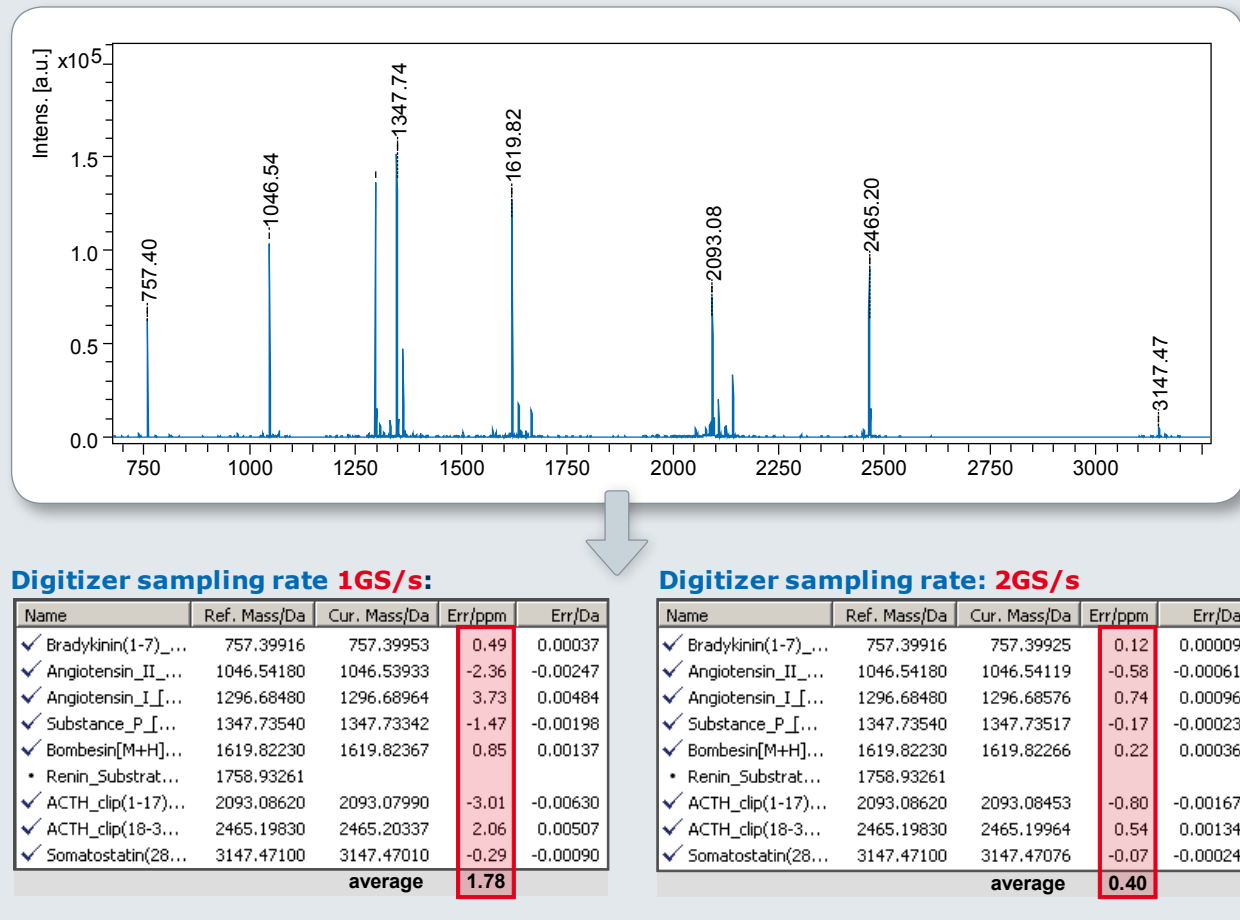


Fig. 3: Impact of the digitizer sampling rate on the achieved mass accuracy: Applying a digitizer rate of 2GS/s in the detection of high resolution peptide signals ( $R \geq 40,000$ , see Fig. 2) results in significantly improved mass accuracy, compared to detection at 1GS/s, due to the increased number of data points describing the spectral peaks.

### Enhanced protein and peptide identification rate

MS/MS data quality represents another important aspect, where the ultrafleXtreme provides significant improvements. Figure 5 displays the MALDI-TOF/TOF spectrum of precursor peptide  $[M+H]^+ = 1984.037\text{Da}$ , which was identified as peptide sequence GMGPENTLILIDGKPVSSR originating from Ferrienterobactin receptor. The spectrum shows clear isotopic resolution across the entire fragment mass range. The mass error averaged over all matching fragments was well below 0.05Da.

While the example MS/MS given in Figure 5 represents a highly abundant peptide, Figure 6 shows the annotated MS and MS/MS spectra obtained from a low abundance precursor ( $S/N=10$ ). As a good illustration of the ultrafleXtreme's MS/MS sensitivity, this minor compound

was unambiguously identified as peptide sequence CDMVDDEELLELVEMEVK from Elongation factor Tu, based on an abundant  $\gamma$ -ion series.

The overall identification results obtained from the LC-MALDI analysis of 500ng *E.coli* digest are summarized in Table 2. 775 proteins were identified applying MASCOT standard stringency settings ( $p < 0.05$ ). Enhancing the stringency of result filtering to a global peptide FDR of 1%, 664 proteins remained identified indicating the high significance of the dataset. 40% of all MS/MS spectra (a total of 3877) were assigned to tryptic peptides considering a profile of 11 modifications. Taking into account also semi-trypsin and non-trypsin peptides, nearly 50% of all acquired MS/MS spectra (4595 in total) could be assigned to peptide sequences originating from *E.coli* proteins.

## ProteinScape: Organizing Proteomics Data

Complex samples yield large amounts of data which need to be organized in a way that mirrors the workflows utilized in the lab. Furthermore, easy access to data on global level as well as single spectra level is necessary. ProteinScape has been developed with particular attention to these requirements. Figure 7 shows the graphical user interface of ProteinScape when working with LC-MALDI data. All details featured by the dataset (protein level, peptide level, chromatographic perspective, single MS and MS/MS spectra) are accessible by a single mouse click. To ensure high processing speed even for extremely large datasets, ProteinScape handles peaklists instead of raw data.

However, raw data is available on demand at any time along the data analysis workflow. ProteinScape's extensive query functionalities provide easy access to specific biological or analytical information from the database. Figure 8 shows part of the result table obtained from a peptide query searching the *E.coli* dataset for assigned tryptic peptides representing acetylated protein N-termini.

Another essential software feature is the implemented ProteinExtractor™ algorithm. This algorithm allows compilation of non-redundant protein lists from multidimensional experiments involving multiple workflows, multiple instrument platforms and multiple search engines.

### Low ppm mass accuracy on a complex *E.coli* sample

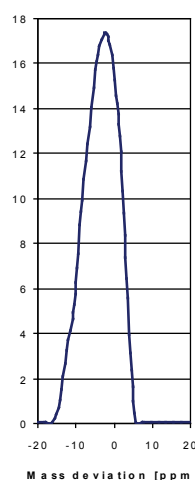
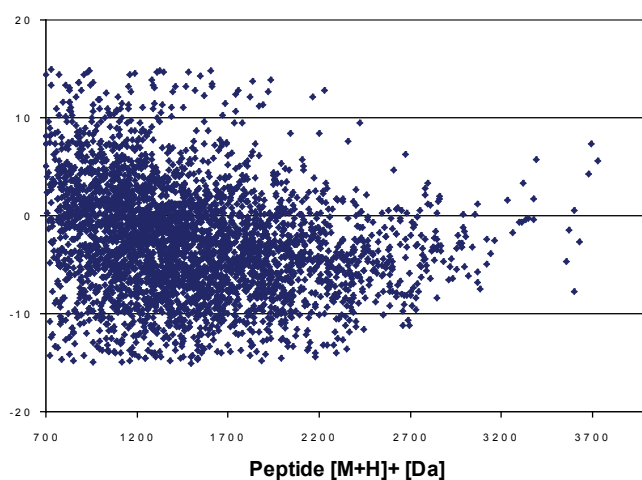


Fig. 4: Distribution of mass errors plotted for all peptides identified from nanoLC-MALDI-TOF/TOF analysis of 500ng *E.coli* digest performed on the ultrafleXtreme. The mass error averaged over all matching peptides was 4.98ppm (external calibration). 90% of all peptide matches were within 10ppm, 56% within 5ppm, and 36% were detected with less than 3ppm mass deviation.

**Table 2:**

### Summarized result obtained from nanoLC-MALDI-TOF/TOF analysis of 500ng *E.coli* digest performed on the ultrafleXtreme

<b>No. of acquired LC fraction MS spectra</b>	768
<b>Acquisition time per MS spectrum</b>	3s
<b>No. of detected peptide compounds (S/N&gt;3)</b>	18450
<b>No. of acquired MS/MS spectra (parent S/N&gt;7)</b>	9753
<b>Acquisition time per MS/MS spectrum</b>	≤5s
<b>No. of identified proteins (p&lt;0.05)</b>	775
<b>No. of identified proteins (global peptide FDR 1%)</b>	664
<b>No. of assigned tryptic peptides</b> (≤1 partial; variable modifications: Ox (M), CAM(C))	3467
<b>No. of assigned tryptic peptides</b> (≤1 partial; extended profile of 11 variable modifications)	3877
<b>No. of assigned peptides</b> (extended profile of 11 variable modifications, including semi-tryptic and non-specific peptides)	4595

### Clear isotopic resolution across entire fragment mass range

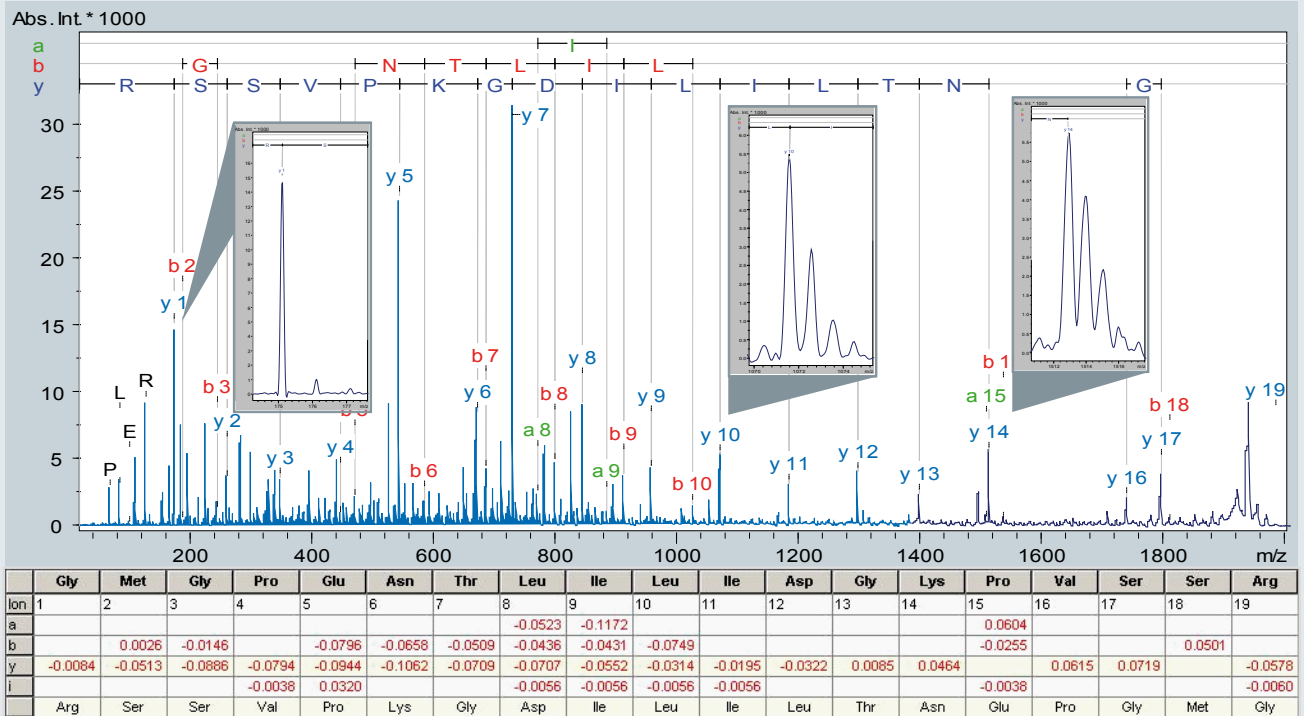


Fig. 5: MALDI-MS/MS spectrum obtained from a peptide (GMGPENTLILIDGKPVSSR,  $[M+H]^+=1984.037\text{Da}$ ,  $t_R=77.3\text{min}$ ), identified from Ferrierterobactin receptor (*E.coli*). The ultrafleXtreme provides isotopic resolution over the entire range of peptide fragments. Mass error averaged over all matching fragments was  $0.045\text{Da}$

### ID of a low abundant precursor

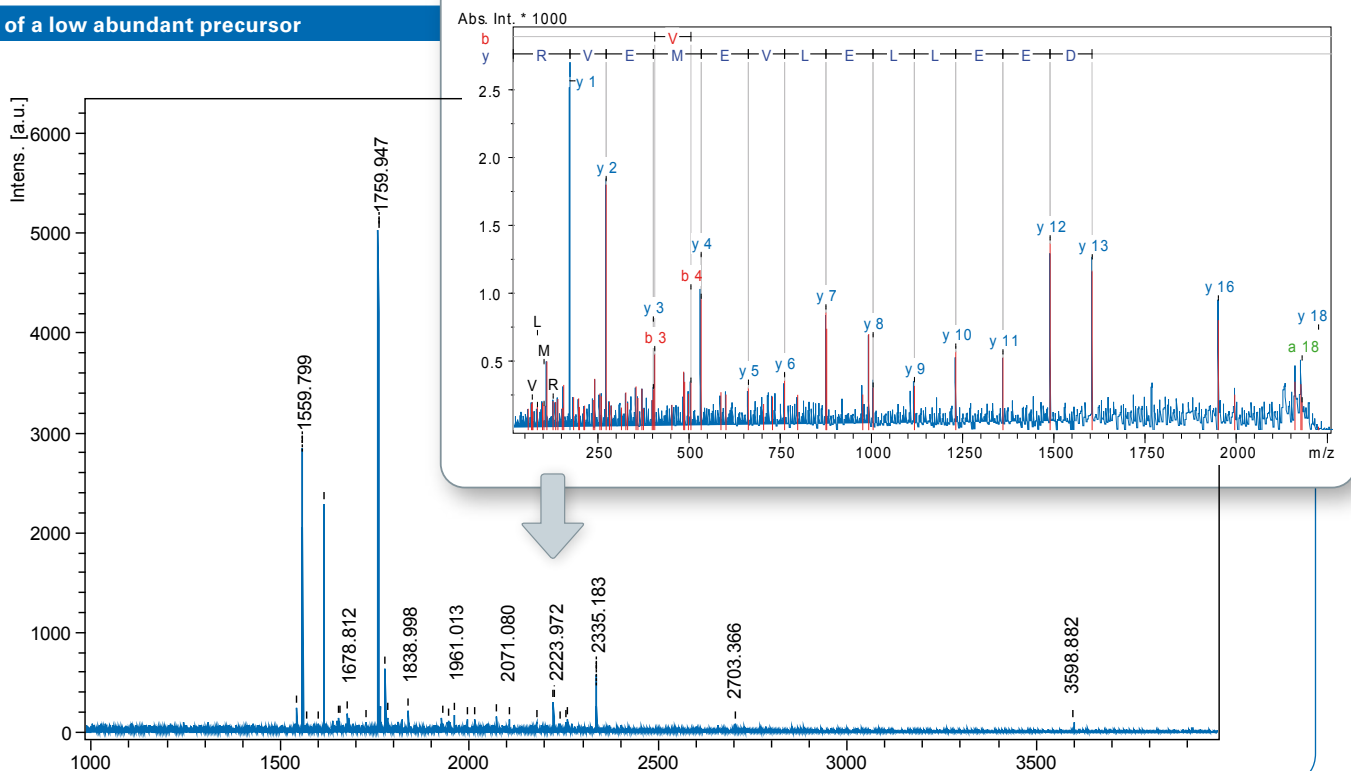


Fig. 6: High quality MS/MS data obtained from low abundance peptide compounds: The displayed MS/MS spectrum was generated from a low abundance peptide ( $S/N=10$ , parent  $m/z = 2223.972\text{Da}$ ) eluting at retention time  $t_R=108.8\text{min}$ . Based on an abundant y-ion series, the peptide was unambiguously identified as CDMVDDEELLELVEMEVR from Elongation factor Tu (*E.coli*).

## Summary

With the introduction of Bruker's ultrafleXtreme instrument, a new, so far unobtained level of MALDI-TOF/TOF performance is available. Resolution of  $\geq 40,000$ , low to sub-ppm mass accuracy and low attomol sensitivity translate directly into a tremendous increase in the number of proteins and peptides identified with confidence from complex proteomics samples. kHz acquisition speed, available on the ultrafleXtreme in both MS and MS/

MS mode, allows the generation of dramatically more information from biological samples within significantly shorter time. Interfacing the ultrafleXtreme to the ProteinScience proteomics database system, large quantities of MS and MS/MS data are organized, analyzed in-depth and archived with ease. These unique features make the ultrafleXtreme the first choice of researchers who are facing the challenges of life science without making compromises.

### Easy access to all dataset details with ProteinScience

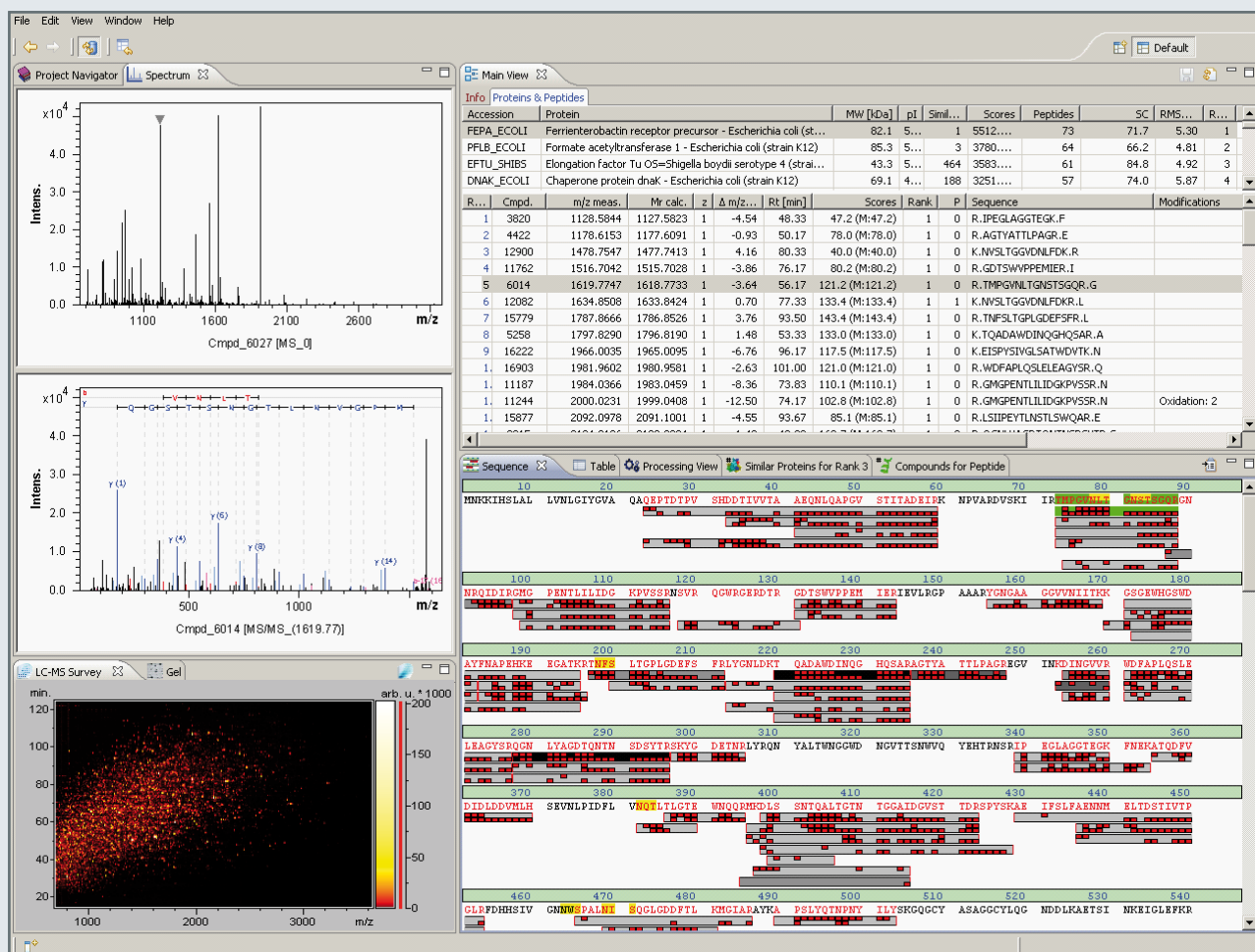


Fig. 7: Data analysis performed in Bruker's unique ProteinScience database software: The software allows organization of large scale proteomics data in a way that mirrors exactly the experimental workflows applied throughout a particular project. The software interface, nevertheless, provides easy access to the archived data down to the level of single spectral items, including rawdata for in-depth evaluation.

## ProteinScape peptide query functionality

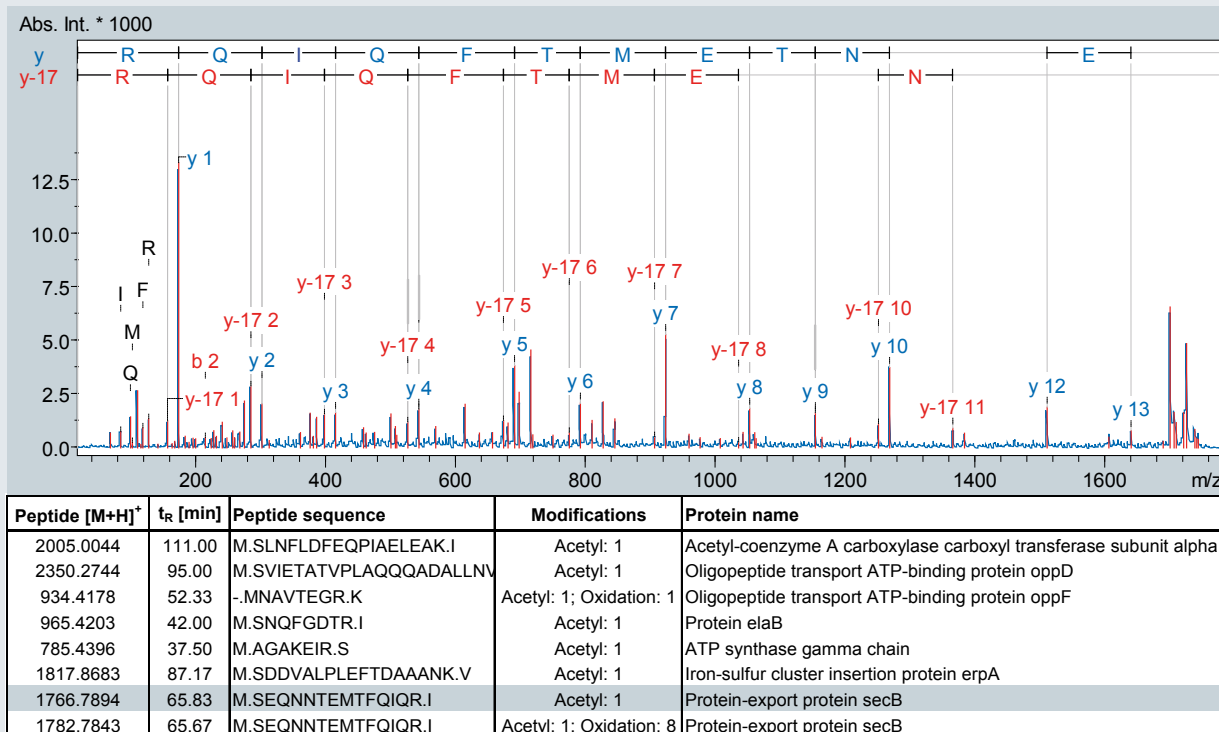


Fig. 8: Query functions in ProteinScape ensure instant access to specific biological or analytical information derived from large datasets: The figure shows part of the result obtained from a query performed on the *E.coli* LC-MALDI dataset to trace selectively tryptic peptides representing acetylated protein N-termini. For the highlighted peptide entry, the corresponding MS/MS spectrum is displayed in the upper part of the figure.

## References

- [1] ultrafleXtreme – Redefining MALDI-TOF/TOF performance, Bruker Technical Note TN-31
- [2] A. Holle, A. Haase, M. Kayser, J. Höhndorf, Optimizing UV laser profiles for improved MALDI performance, *J. Mass Spectrom.* 2006; 41; 706-716

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## Keywords

LC-MALDI  
MALDI-TOF/TOF  
nanoLC  
Protein identification  
Resolution  
Mass Accuracy  
Analysis Speed  
*E.coli*  
Sample complexity

## Instrumentation & Software

ultrafleXtreme  
EASY-nanoLC  
PROTEINEER fc II  
MTP AnchorChip 800-384  
ProteinScape

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