

Kindly provided by Christian Dusny, Helmholtz-Centre for Environmental Research, Leipzig, Germany

## ● MRMS powered single cell metabolomics – Quantification of picogram amounts of a biocatalytic product from few living cells

Single cell metabolomics via mass spectrometry can successfully cope with many related analytical requirements such as very small volumes and low analyte concentrations typically found in intra- and extracellular media.

### Abstract

Probing the metabolic state of **living** microbial cells via their secreted metabolic products is highly challenging as it requires measures to maintain the

cellular integrity and to collect the analytes. This application note demonstrates how direct infusion magnetic resonance mass spectrometry (DI-MRMS) is able to quantify lysine produced by a few intact microbial

cells. The high mass resolution and sensitivity of MRMS in combination with a reliable sample introduction and transfer is a helpful tool for assessing cellular processes.

*Keywords:*  
solarIX, single cell analysis, metabolomics, MRMS

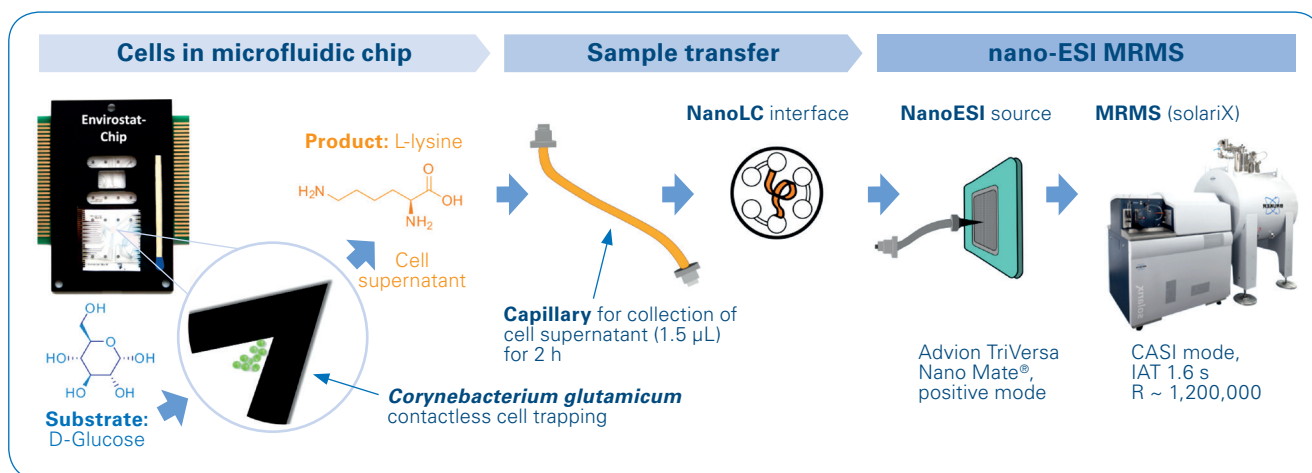


Figure 1: Workflow for the analysis of single cell secretome using MRMS [1].

## Introduction

Information on the metabolic state of individual cells in a population can't be generated from a bulk analysis of the culture. Microfluidic systems are powerful tools to study living single cells. These miniaturized bioreactors enable the precise control, growth, and monitoring of single cells [1]. While optical methods to study single cells are well advanced, mass spectrometric applications are mostly limited to destructive imaging techniques such as MALDI. To better understand the metabolic activity and metabolite productivity of a living cell, label-free analytical concepts for the detection of minute amounts of secreted low molecular weight compounds are needed.

Here, we report an analytical framework that interfaces non-invasive microfluidic trapping and cultivation of a few bacterial single cells (*Corynebacterium glutamicum* DM1919 pSenLys) with the quantification of their catalytic products by DI-MRMS (Figure 1) [1].

The main challenges of this framework are:

- Transfer of a small sample volume (~1 µL) to the ion source

- Detection of secreted metabolites at low concentration (pg-ng cell<sup>-1</sup> h<sup>-1</sup>)
- Matrix effects caused by the buffer and possible isobaric/isomeric interferences.

## Methods

### Cell sampling and transfer of µL-sample volumes

Cell populations of *C. glutamicum* DM1919 were cultivated using CGXII medium, harvested, washed, and transferred to the microfluidic Envirostat chip [2]. Microfluidic cultivations with the Envirostat were performed in glucose-containing ammonium bicarbonate (ABC) buffer to facilitate subsequent direct infusion ESI analysis. Cell supernatants from Envirostat cultivations (i.e. medium with the secreted product lysine) were collected in 1.5 µL PEEK-capillaries (Waters Corporation, USA). After 12 h of incubation, the capillary was removed, sealed with PEEK-MicroTight adapters and immediately transferred to the MRMS for mass spectrometric analysis (Figure 2).

### nanoESI-DI-MRMS measurements

All mass spectrometric measurements were performed with a 12 T solariX XR MRMS system. A nano-ESI ion source (TriVersa Nanomate, Advion

BioSciences) was used in positive ion mode (dry gas flow: 8 L/min, dry temperature: 150°C, capillary voltage: 1.7 kV). An LC-coupler (20 cm fused silica capillary, Advion BioSciences) was connected to a six-way auto-sampler valve of a NanoLC system (Ultimate 3000 nanoRSLC, Thermo Fischer Scientific) via a nanoViper-capillary (ID: 20 µm, OD: 1/16", length: 750 mm, Thermo Fischer Scientific). LC-Coupler and nanoViper capillary were connected with a PEEK-MicroTight adapter (P-882, IDEX Health & Science LLC). The cell supernatant was transferred into a 1 µL nanoViper sample loop and subsequently transported to the nanoESI source with a flow rate of 150 nL/min. A solution of 1:1 (v/v) ultrapure water and methanol, supplemented with 0.1% (v/v) formic acid was used as eluent.

CASI mode (Continuous accumulation of selected ions) was used at  $m/z$  147.5 with a window size of  $\pm 2.5$  Da centering the quasimolecular ion of lysine ( $[M+H]^+$ ,  $m/z$  147.112804) and its isotopologue. Mass spectra were recorded in the mass range  $m/z$  73–1000 and time of flight of 0.6 ms using broadband mode detection. Data were processed in magnitude mode with data size 8 MW (transient length of 3.5 s). 64 single scans were co-added with an ion accu-

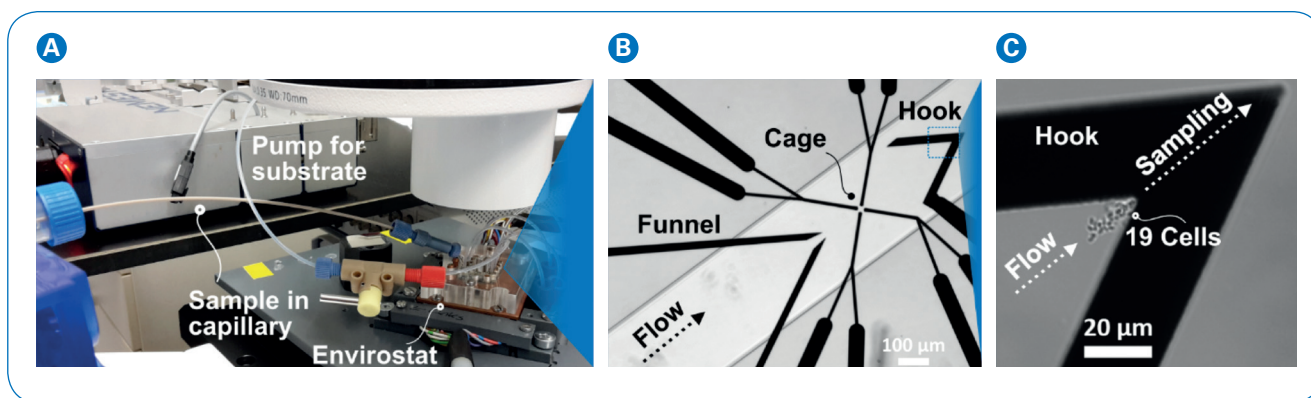


Figure 2: (A) Setup for sampling the secretome from a few microbial cells. (B) Flow path inside the Envirostat chip: in the microfluidic channel cells are focused in the funnel and can be captured either in the cage or in the hook. (C) Zoom into the region around the hook: 19 cells are captured contactless by negative dielectrophoresis while the secreted metabolites were sampled for 2 h [1].

mulation time (IAT) of 1.6 s per scan. Peaks were picked when their signal-to-noise (S/N) ratio was greater than four. Peak intensities were used for quantification. Instrumental limits of detection and quantification were determined by taking mean peak intensities plus 3× and 9× the respective standard deviation of repeated blank measurements.

As internal standard,  $^{15}\text{N}_2$ -labeled L-lysine (Sigma-Aldrich) was used. For method blank assessment, PEEK capillaries were filled at the outlet of the Envirostat under cultivation conditions (before cell transfer) and injected as described above.

## Results

### Transfer of $\mu\text{L}$ -Volumes to MRMS

The transfer of  $\mu\text{L}$  sample volumes to a mass spectrometer is routinely performed with LC-systems and appropriate autosamplers and vials. However, this procedure is not feasible for small total available sample volumes of 1  $\mu\text{L}$  and less. The injection-system would require more volume than a single cell sampling approach would deliver, thus wasting valuable sample. Sampling of a microfluidic bioreactor (Envirostat) in vials bears the risk of sample contamination, and solvent evaporation, thus

changing metabolite concentration. In contrast, the sampling of  $\mu\text{L}$ -volumes of secretome samples in PEEK-capillaries allows for a direct hyphenation to the Envirostat and storage of the sample overnight. Directly, connecting the capillary to a nanoLC injection valve is a functional setup for the lossless transfer of the 1.5  $\mu\text{L}$  secretome samples (Figure 3). A reliable aliquotation and injection of 1  $\mu\text{L}$  of the sample was possible using the autosampler valve and syringe.

Since the focus was on the analysis of a highly polar, basic amino acid (i.e. lysine) direct-infusion MRMS provided an “unbiased view” into the sample without altering the composition of the sample. However, the sensitivity of the analysis was limited by a background signal on the exact mass of lysine. To limit the effect of the blank, the application of a (nano)-LC-separation could be beneficial.

Since a volatile buffer was used for cell cultivation (ammonium bicarbonate) desalting of the sample before analysis was not necessary.

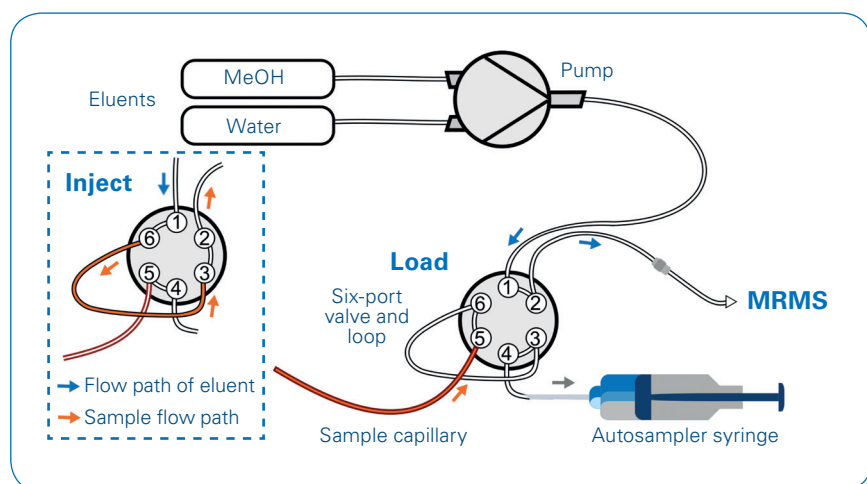


Figure 3: Setup for the injection of  $\mu\text{L}$  volumes via a nanoLC-system from a capillary

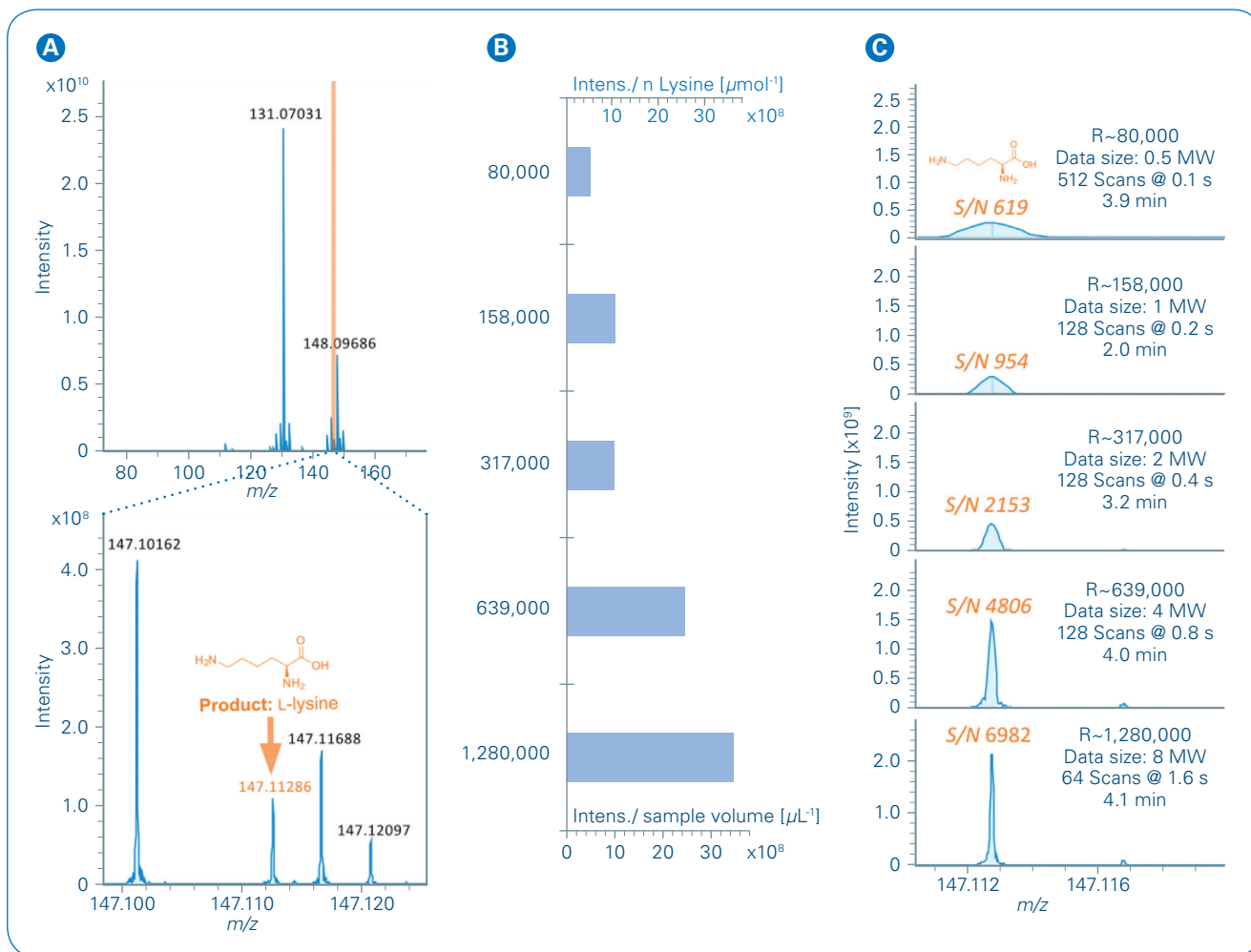


Figure 4: **(A)** Top: CASI spectrum of a secretome sample from 19 cells ( $m/z$   $147 \pm 2.5$  Da). Bottom: Zoom into the detected mass of lysine ( $[M+H]^+$ , mass error: 0.37 ppm). **(B)** The intensity of the signal of lysine normalized to the consumed sample volume and the amount of substance needed for generating the spectrum for different transient length. **(C)** CASI spectrum of a lysine standard (70 ng/mL,  $m/z$  147.11280) for different transient length.

#### Effect of transient length and accumulation time on sensitivity

The benefit of ultra-high mass resolution MRMS can be observed on the nominal mass of lysine (Figure 4A). The mass peak of lysine is fully resolved despite the complexity of the sample - laying the foundation of reliable quantification of this metabolite. Additional improvement in the sensitivity can be achieved by increasing the ion accumulation time (IAT) after CASI isolation and the co-addition of 64 single scans for a final mass spectrum. However, this approach is often limited by ion-

gas and ion-ion interactions (e.g.  $H_2O$  loss as observed in Figure 4A for some co-isolated masses) in either the collision cell or analyzer cell, reducing the number of ions for detection and deteriorating the mass peak shape. Also the available sample volume (max. 1.5  $\mu$ L) was limiting the total analysis time and the nanoESI source was chosen to maximize the time available for spectra acquisition. In order to increase the overall duty cycle of the instrument the software feature 'accumulate ion during detection' was used. For an optimal analysis of a  $\mu$ L-sample volume, we compared

different transient length and IAT settings for a given maximum time of analysis of 6.5 min (1  $\mu$ L sample volume @ 150 nL  $min^{-1}$  infusion rate). We found that the combination of a very narrow isolation window ( $m/z$   $147 \pm 2.5$  Da), high IAT (1.6 s) and long transients of 3.5 seconds (resulting in highest S/N) provided highest sensitivity for the analysis of individual metabolites in small sample volumes (Figure 4B and C). Instrumental limits of detection and quantification (from 1  $\mu$ L sample volume) were determined as 0.5 and 1.1 ng/mL, respectively. This limit of detection corresponds to just 1.1 pg or 7.5 fmol of lysine.

Quantification of a metabolic product from ca. 19-21 cells

With the developed setup of sample transfer and highly sensitive detection of lysine from microliter sample volumes, the application to single cell analysis is within reach. Since the overall time to generate a single replicate sample was in the order of 24h, we used an isotope labelled internal standard ( $^{15}\text{N}_2$ -lysine) to compensate for day-to-day variability. The internal standard was added directly to the Envirostat medium.

Calibration of our MRMS method was done via injection of standards (plus internal standard) from vials and a full method blank was generated daily from Envirostat supernatants prior loading of the cells (Figure 2 and Figure 5).

We could successfully quantify the produced and secreted lysine from 19 (8.5 ng/mL) and 21 cells (38.8 ng/mL). This enabled us, for the first time, to assess the specific productivity of lysine between 2 and 10  $\text{fmol cell}^{-1} \text{h}^{-1}$  -calculated from just a few bacterial cells.

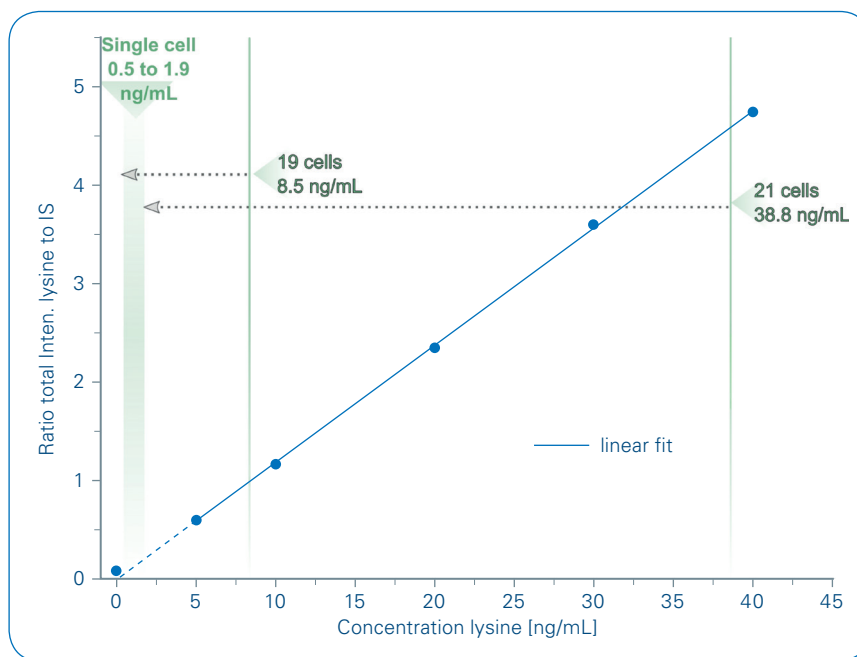


Figure 5: Calibration of lysine using the nanoLC-nanoESI-MRMS setup. Duplicated injection (mean values shown) of standards from vials with the respective linear fit are shown. Samples were prepared in 20 mM ABC buffer with 500 mM glucose. The internal standard (IS) concentration was 10 ng/mL and the lysine calibration range was adjusted to the expected concentrations from the experiments with cells [1].

## Conclusion

- Direct infusion MRMS can be used for the analysis of metabolites in very small sample volumes.
- A stable sample flow and nano-electrospray is important for a reliable quantification of metabolites at the single cell level.
- MRMS provides benefits for complex metabolomic samples: high sensitivity and ultrahigh mass resolution for separation of isobaric signals.
- Detection and quantification of other, potentially more sensitive, low blank probes for cell metabolism are possible (by simply adjusting the CASI window).



## Learn More

You are looking for further Information?  
Check out the link or scan the QR code for more details.

[www.bruker.com/solarix](http://www.bruker.com/solarix)



### References

- [1] Dusny C, Lohse M, Reemtsma T, Schmid A, Lechtenfeld OJ (2019). *Quantifying a Biocatalytic Product from a Few Living Microbial Cells Using Microfluidic Cultivation Coupled to FT-ICR-MS*. *Anal. Chem.*, **91**, 7012–7018.
- [2] Rosenthal K, Falke F, Frick O, Dusny C, Schmid A (2015). *Micromachines*, **6** (12), 1836–1855. <https://www.mdpi.com/2072-666X/6/12/1459>

For Research Use Only. Not for Use in Clinical Diagnostic Procedures.

### ● Bruker Daltonik GmbH

Bremen · Germany  
Phone +49 (0)421-2205-0

### Bruker Scientific LLC

Billerica, MA · USA  
Phone +1 (978) 663-3660

[ms.sales.bdal@bruker.com](mailto:ms.sales.bdal@bruker.com) – [www.bruker.com](http://www.bruker.com)