FlashNote

iprm-PASEF in Action: A transformative solution for untargeted spatial metabolomics

Bruker's integrated MALDI-TIMS-MS2 workflow empowers researchers to annotate natural products directly from co-cultures with unprecedented speed and precision. The approach mitigates the need for orthogonal workflows, accelerating discovery in spatial metabolomics.

Why it matters

MALDI Imaging is a powerful technique for visualizing the spatial distribution of metabolites in biological samples. However, microbial imaging faces challenges due to sample complexity, low ion abundance, and limited confidence in MS1-level annotations. The newly developed imaging parallel reaction monitoring–parallel accumulation serial fragmentation (iprm-PASEF®) workflow addresses these limitations by integrating trapped ion mobility spectrometry (TIMS) and MS2 acquisition directly into MALDI Imaging [1]. This enables rapid, high-confidence molecular annotation directly from microbial colonies, and in some cases, removes the need for orthogonal validation techniques like LC-MS or NMR.

Methods

In this study, iprm-PASEF was employed to investigate microbial metabolite production in a co-culture of *Glutamicibacter arilaitensis* and *Penicillium solitum*. The experiment leveraged Bruker's timsTOF fleX platform and SCiLS™ Lab software to perform targeted MS2 acquisition directly from agar-grown cultures. Using SCiLS Lab's statistical tools, 12 precursors were selected for iprm-PASEF acquisition, enabling multiplexed imaging and high-throughput MS2 data collection. The workflow integrated TIMS-based mobility separation with parallel reaction monitoring, allowing for spatially resolved, mobility-filtered MS2 spectra to be exported and annotated using GNPS2, MetaboScape®, and SIRIUS, all without the need for additional cultivation or LC-MS workflows. Further methodological details can be found in the referenced literature [2].

Outcome

This study highlights the impact of iprm-PASEF technology in enabling rapid and confident annotation of microbial natural products directly from complex co-culture samples. By combining TIMS with MS2, researchers achieved isomer-level resolution and accurately identified coproporphyrin III, an annotation that previously required labor-intensive methods such as HPLC and NMR [3].

With iprm-PASEF, the entire analysis was completed in under three hours, dramatically reducing time and resource investment. The vendor-supported workflow, including SCiLS Lab's automated precursor selection and export tools, simplifies the entire process from acquisition to annotation. This positions iprm-PASEF as a transformative solution for untargeted spatial metabolomics and accelerates natural product discovery in microbial systems.

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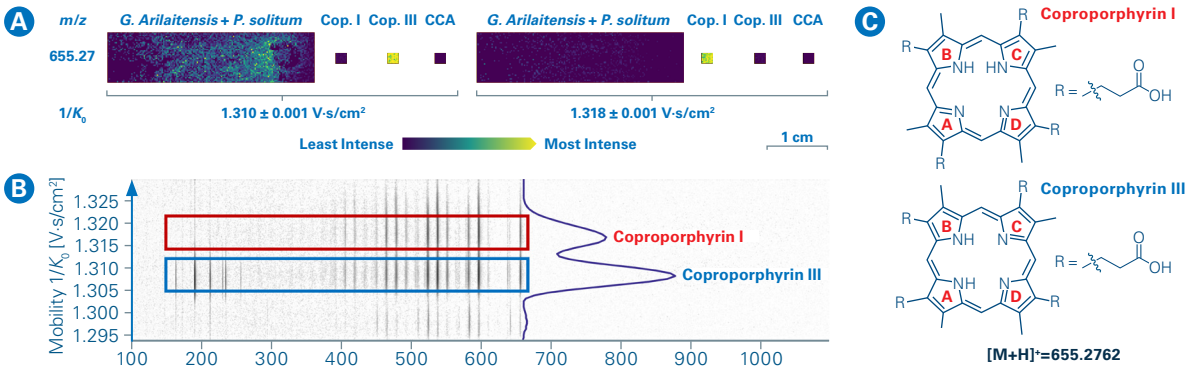

Figure 1. Confirmation of coproporphyrin III in co-culture using iprm-PASEF. (adapted from Shepherd et al. [2])
Ⓐ Ion images for the major fragment of coproporphyrin (*m/z* 655.27), resolved solely by ion mobility ($1/K_0$). Fragments detected in co-culture align with the mobility range of the coproporphyrin III standard and match previously reported spatial distributions of coproporphyrin III produced by *G. arilaitensis* in co-culture. Ⓑ Mobility-resolved spectra highlighting coproporphyrin I (red) and III (blue). Ⓒ Chemical structures of coproporphyrin I and III. Key abbreviations: "Cop" = coproporphyrin; "CCA" = cheese curd agar.

Key Highlights

- **Rapid turnaround:** Complete MS2-based annotation in under 3 hours, removing the need for additional culturing or LC-MS/NMR validation for this compound class.
- **High-confidence identification:** TIMS-enabled isomer resolution allows accurate annotation of coproporphyrin III directly from co-culture.
- **Smart precursor selection:** SCiLS Lab's statistical tools streamline untargeted feature prioritization and reduce matrix interference.
- **Integrated workflow:** From acquisition to export, all steps are supported by Bruker's commercial software, no third-party tools required.
- **Scalable for discovery:** Enables untargeted spatial metabolomics in complex microbial systems, accelerating natural product research.

For more information see the original publication "*MALDI-TIMS-MS2 Imaging and Annotation of Natural Products in Fungal-Bacterial Co-Culture*", published by Shepherd et al. [2].

References

[1] Technical Note TN-60

[2] Shepherd RA, Luu GT, Sanchez LM (2025). *MALDI-TIMS-MS2 Imaging and Annotation of Natural Products in Fungal-Bacterial Coculture.* Anal Chem. **97**, 35, 18867-18872. https://doi.org/10.1021/acs.analchem.5c02787

[3] Cleary JL, Kolachina S, Wolfe BE, Sanchez LM (2018). *Coproporphyrin III Produced by the acterium Glutamicibacter arilaitensis Binds Zinc and Is Upregulated by Fungi in Cheese Rinds.* ASM Journals. **3**, 4. https://doi.org/10.1128/msystems.00036-18

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"What once took over a week of culturing, extraction, and multi-platform validation can now be done from single colonies in just three hours. With iprm-PASEF, we've turned a labor-intensive workflow into a rapid, high-confidence annotation pipeline—unlocking new speed and precision for spatial metabolomics in complex microbial systems."

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