

# Enveda Biosciences uses TIMS and machine learning to accelerate natural product drug discovery



A world of unidentified natural products is waiting to be discovered in the complex chemical diversity of medicinal plants. Enveda Biosciences goes beyond untargeted metabolomics to identify novel therapeutics with machine learning of CCS and MS/MS data coupled with bioassay information.

TIMS provides unambiguous CCS and MS/MS data that is ideal for natural product analysis, as demonstrated by Enveda Biosciences.

## Challenge

Natural product drug discovery is a challenging process, often hampered by the difficulty of isolating and identifying bioactive compounds from complex mixtures. Traditional methods rely on bioactivity-guided fractionation, in which the natural product extract is tested for bioactivity in a targeted biological experiment. If the extract shows strong bioactivity, it is fractionated using chromatography to separate the mixture into its constituent parts. Each fraction is then tested again in the bioassay to identify the active component. This process is repeated until the active compound is isolated.

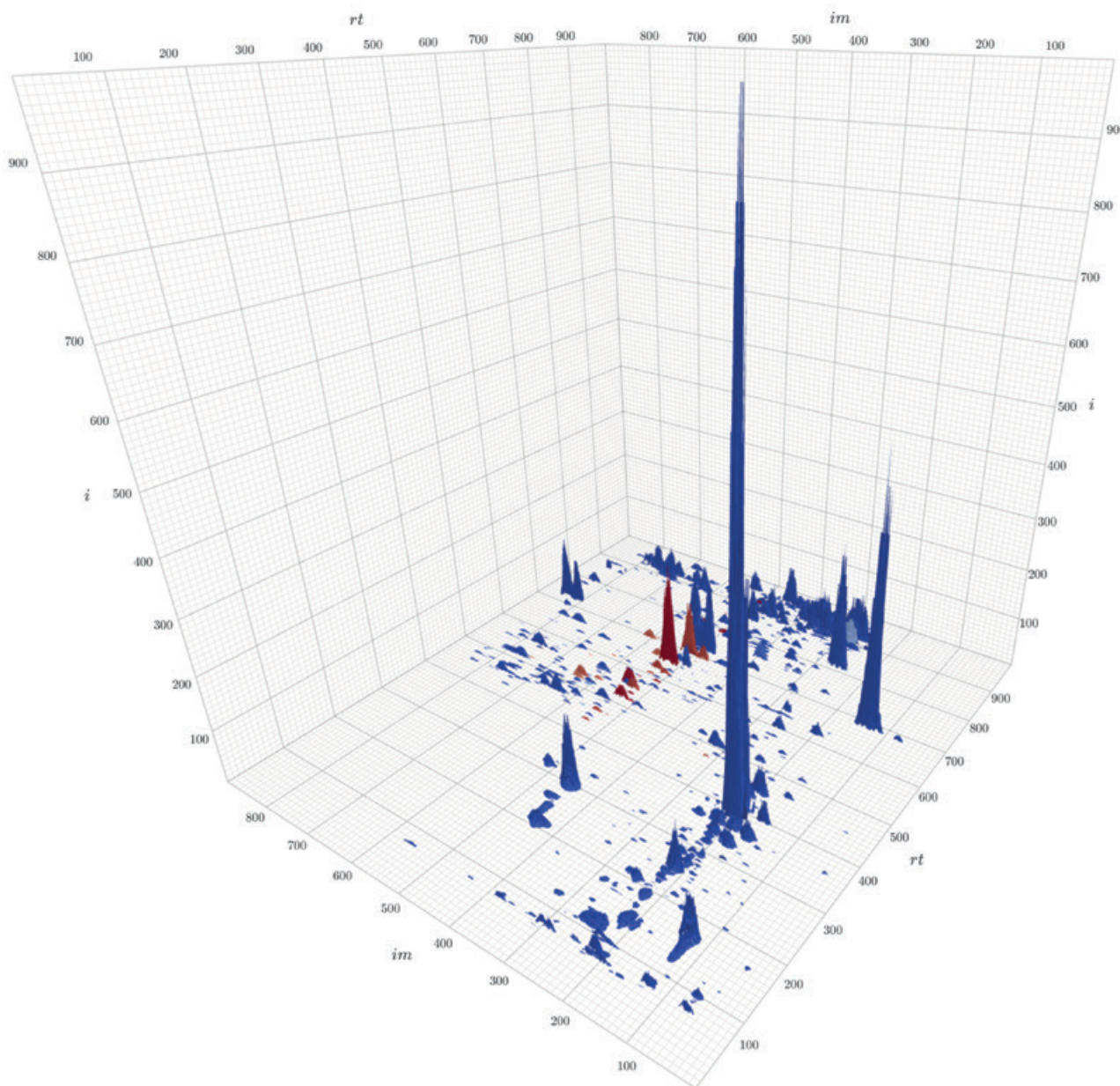
### Bioactivity-guided fractionation has several limitations:

- It is time-consuming and inefficient, requiring repeated cycles of separation and testing.
- It can be difficult to identify rare or low-abundance compounds.
- The dilution effect of fractionation can impact the efficacy of the “hit” compound in subsequent bioassays.
- Cumulative or synergistic effects from multiple bioactive species may be lost during fractionation, making it challenging to identify relationships between secondary metabolites that contribute to bioassay efficacy.

Despite these limitations, bioactivity-guided fractionation remains a valuable tool for natural product drug discovery. However, new techniques are emerging that can help to address these limitations.

## Solution

It takes a long time to identify unknown components in natural products using the standard combination of mass spectrometry (MS) and nuclear magnetic resonance spectroscopy (NMR). Enveda Biosciences is taking natural product drug discovery to the next level by probing the diverse chemical space of natural products using trapped ion mobility spectrometry (TIMS) and high-resolution quadrupole time of flight (QTOF) MS coupled with their unique computational metabolomics.



**Figure 1**

3D plot of natural product extract analysis on timsTOF Pro 2 visualizing retention time, ion mobility, and peak intensity.

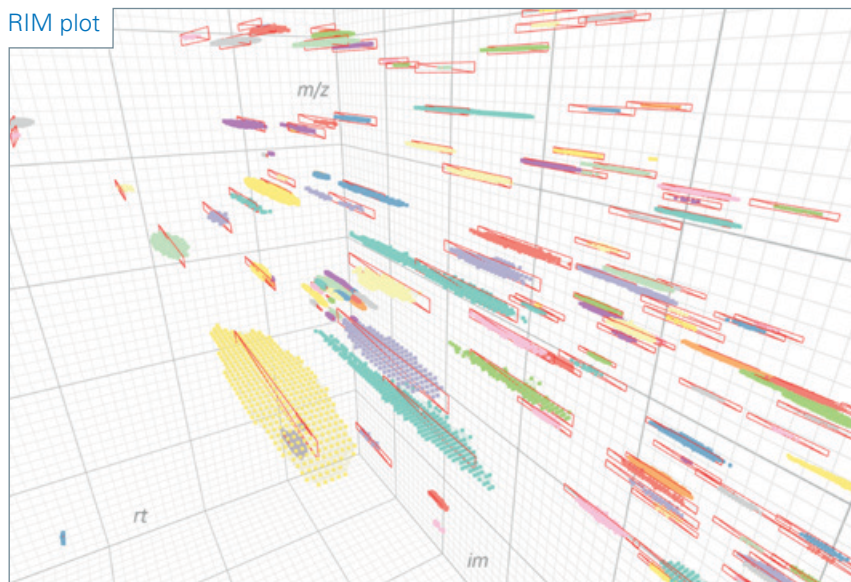
Color gradient represents the cosine spectral similarity of the analyte with a known bioactive compound present in the sample, where red has greater spectral similarity.

## Rapid Drug Discovery

For thousands of years, natural products have been used as therapeutics for a wide variety of maladies, despite the identity of the secondary metabolites being unknown. The development of the pain killer acetylsalicylic acid, a derivative of a natural product from willow tree bark [1] and paclitaxel as a cancer therapeutic from the yew tree [2] are good examples. Small molecule therapeutics are not only identified in this workflow, but the bioactive chemistry of the molecule can be determined.

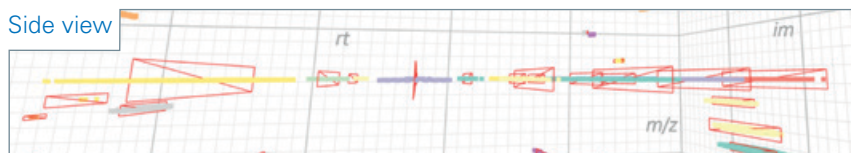
**A**

RIM plot

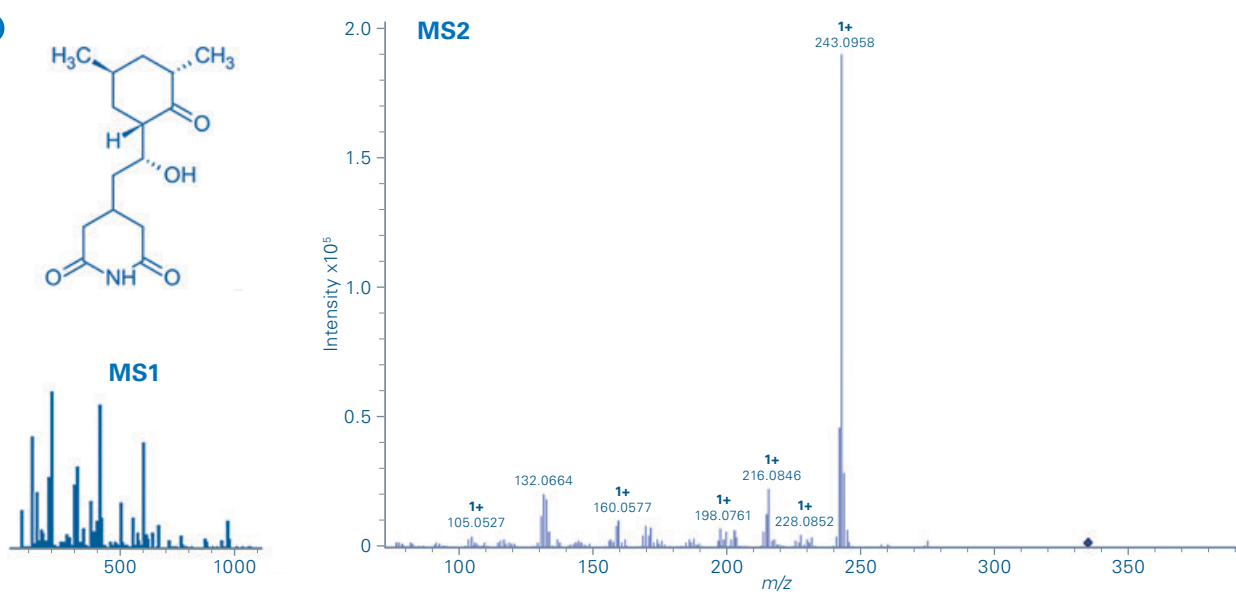


**B**

Side view



**C**



**Figure 2**

**Trapped ion mobility separation of natural product molecules isolated for MS2 fragmentation.**

**A** RIM plot representing retention time (rt) on x-axis, ion mobility (im) on y-axis and  $m/z$  on z-axis. Mobility separated ion packets can be separated, even when they co-elute in the retention time dimension or are isobaric in the  $m/z$  dimension.

**B** Side view zoom highlighting mobility separated precursors isolated for MS2 fragmentation in both the rt and  $m/z$  dimensions.

**C** Example of molecular species isolated from MS1 to a clean MS2 spectrum.

### Trapped Ion mobility and CCS

Enveda utilizes the timsTOF Pro 2 systems to separate molecules based on their size and shape while enabling the measurement of collisional cross section (CCS, Å<sup>2</sup>). The CCS values are a physical property of the molecule and do not change based on the ionization source, giving each measurement a unique characteristic of that molecule.

### Unambiguous MS/MS

TIMS is then coupled with MS/MS fragmentation in an experimental mode called PASEF or parallel accumulation serial fragmentation. As mobility separated ions are "eluted" from the TIMS cell, they are sent to the TOF mass analyzer either directly to collect accurate mass data for the precursor or alternatively fragmented to collect clean mobility-separated MS/MS data.

### Bioassay-driven data analysis

Each MS/MS spectrum is coupled with Enveda's unique computational workflow that involves generative deep learning to understand structural similarity and predict chemical structure. These metabolites are further overlaid with their bioactivity so scientists can focus their power on molecules with the greatest therapeutic interest and most amenable structures.

### Mapping chemical space

The result is rapid pooled characterization of a plant's map of natural product chemical space including bioactivity across target-binding, phenotypic, and protein-protein interaction assays. Using untargeted metabolomics, high-resolution chemical fingerprints can be acquired for thousands of biosamples.



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"In our efforts at Enveda to annotate the structure and function of the natural world, tackling the immense complexity of the natural product space has been made possible by the excellent balance between speed, robustness, resolution, and sensitivity that Bruker's timsTOF platform provides."



### References

- [1] Mehta A (2005). „Aspirin“ Chemical & Engineering News. Vol. **83**, no. 25.
- [2] J Nat Prod. (1990). **53**(5):1249-55. doi: 10.1021/hp50071a017.

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