

# Integrating ion mobility spectrometry and mass spectrometry imaging for characterizing the distribution of biologically active disaccharide isomers

JUNE 1-5 | 2025

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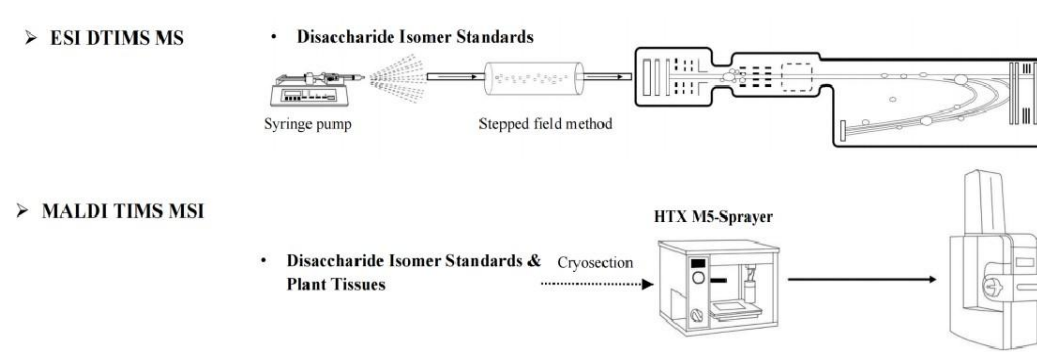
BALTIMORE

SHORT COURSES MAY 31 & JUNE 1

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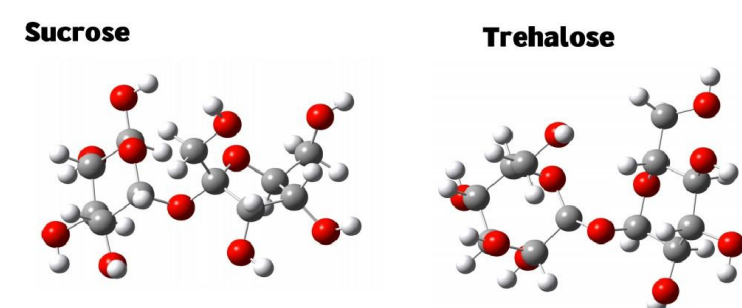
**Introduction:** Sugars are a classic model of isomeric metabolites that widely co-exist within plant tissues and plant-microbe interactions. They serve a variety of biological functions, and revealing their localization and cell-type specificity within tissues can help us better understand their biological roles. While we can visualize the average spatial distribution of mass isomers within a sample with **mass spectrometry imaging (MSI)**, we cannot separate and localize their isomeric complexity.

**Ion mobility spectrometry (IMS)** serves as a powerful strategy for discriminating between and detecting mass isomers. Here, we evaluated both drift tube IMS (DTIMS) and trapped IMS (TIMS) for characterizing sugar isomers, and then we used TIMS-MSI for differentiating and mapping these isomers at near single cell resolution in two model plant systems.



## Novelty

IMS enhanced spatial metabolomics provides a new avenue for characterizing and imaging disaccharide isomers within environmental samples at cellular resolution.

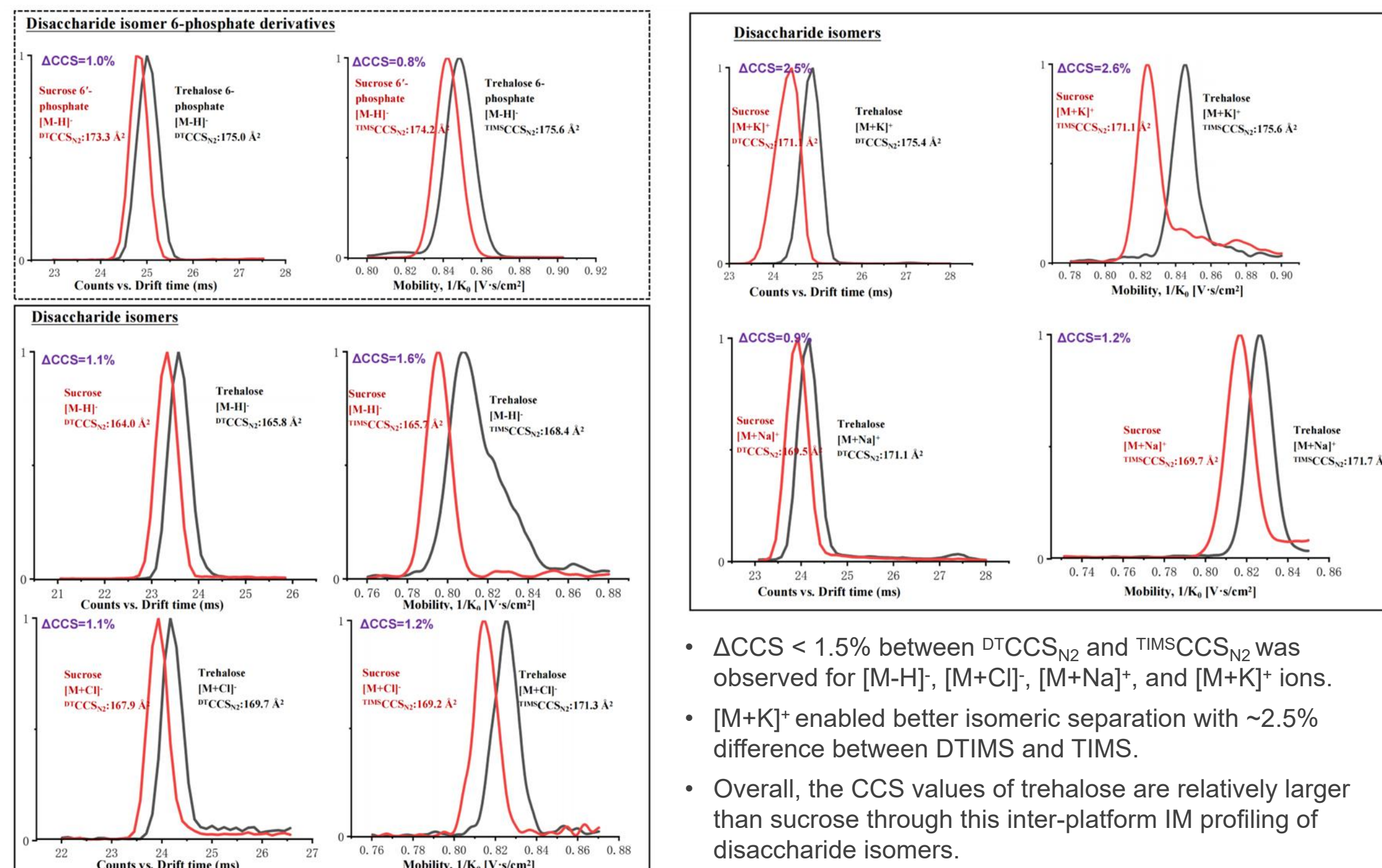


**Workflow:** Disaccharide standards (3  $\mu\text{M}$  in 50% MeOH,  $n = 3$ ) and plant tissues (poplar roots and soybean root nodules) were analyzed. Plant tissues were embedded in 7.5% HPMC/2.5% PVP, cryosectioned at 14  $\mu\text{m}$ , and thaw-mounted onto ITO-coated slides ( $n = 5$ ). DHB and NEDC were applied using an HTX M5-Sprayer for positive and negative ion mode analyses, respectively. Data acquisition was performed using an Agilent 6560 DTIMS-QTOF with a stepped field method and a Bruker timsTOF flex mass spectrometer. IM-MS Browser, DataAnalysis, ImageJ, METASPACE, and SCiLS were used for data analysis.

**Table 1.** Comparison of experimental CCS values measured by DTIMS and TIMS in  $\text{N}_2$ , and empirical or ML-based predicted CCS values. Standard deviation was obtained from 3 replicated measurements.

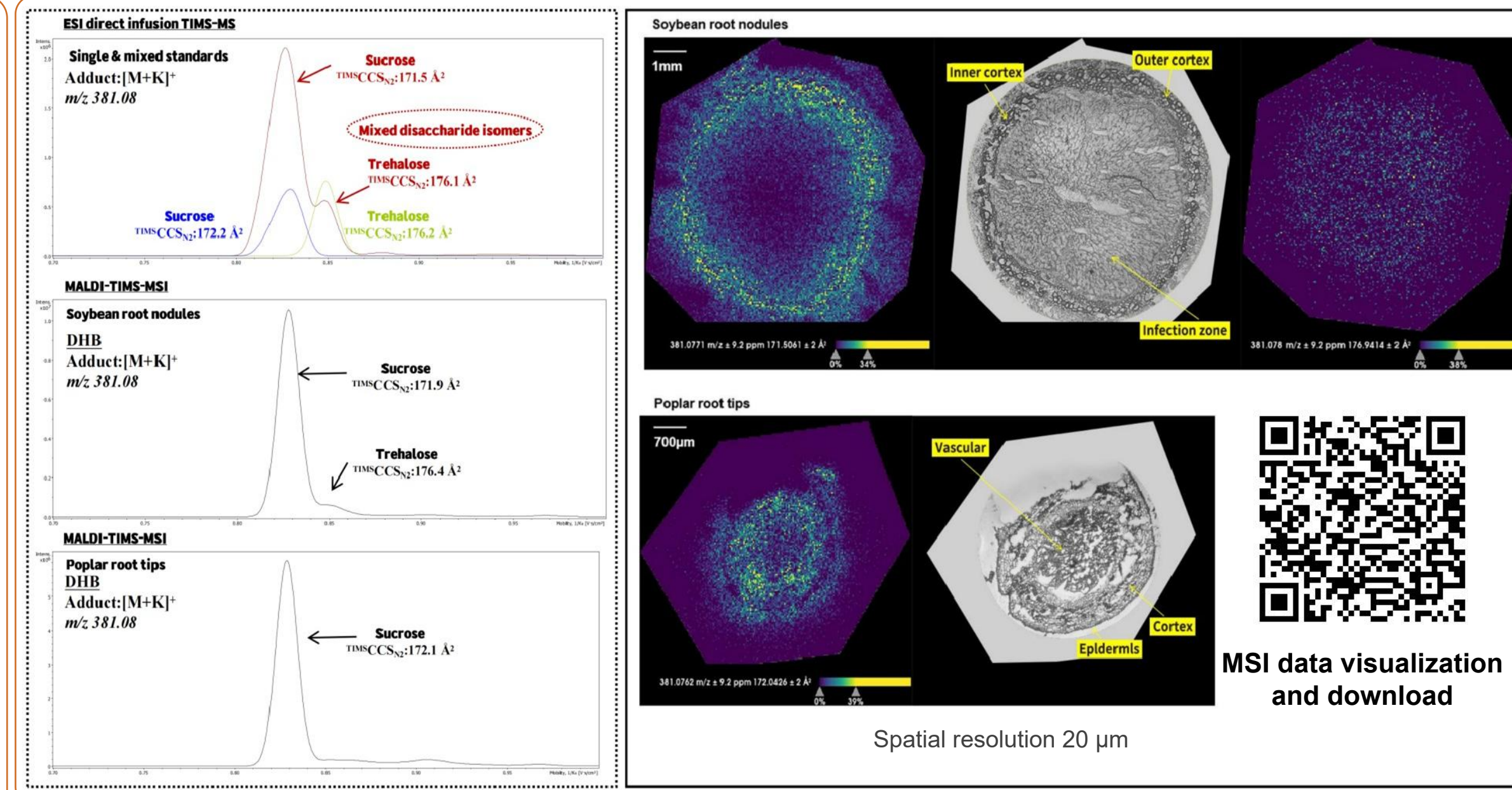
Compounds	$m/z$	Adduct	$^{\text{DT}}\text{CCS}_{\text{N}_2}$ ( $\text{\AA}^2$ )	$^{\text{TIMS}}\text{CCS}_{\text{N}_2}$ ( $\text{\AA}^2$ )	CCS Compendium ( $\text{\AA}^2$ )	Prediction AllCCS ( $\text{\AA}^2$ )	Prediction CCSbase ( $\text{\AA}^2$ )
Disaccharide 6-phosphate isomers	Trehalose 6-phosphate	[M-H] <sup>-</sup>	175.0 ± 0.1	175.6 ± 0.4	180.9 (3.3%; 2.9%)	181.5 (3.4%; 3.3%)	182.8 (4.3%; 3.9%)
	Sucrose 6-phosphate	[M-H] <sup>-</sup>	173.3 ± 0.2	174.2 ± 0.0	-	180.4 (3.9%; 3.4%)	181.2 (4.4%; 3.9%)
Disaccharide isomer	Trehalose	[M+Na] <sup>+</sup>	171.1 ± 0.2	171.7 ± 0.1	176.1 (2.8%; 2.5%)	176.2 (2.9%; 2.6%)	178.0 (3.9%; 3.5%)
		[M+K] <sup>+</sup>	175.4 ± 0.1	175.3 ± 0.1	179.7 (2.4%; 2.4%)	-	179.9 (2.5%; 2.6%)
	[M-H] <sup>-</sup>	165.8 ± 0.2	168.4 ± 0.1	169.9 (2.4%; 0.9%)	169.8 (2.4%; 0.8%)	174.5 (5.0%; 3.5%)	
	[M+Cl] <sup>-</sup>	169.7 ± 0.2	171.3 ± 0.1	-	-	-	
	Sucrose	[M+Na] <sup>+</sup>	169.5 ± 0.2	169.7 ± 0.1	173.9 (2.5%; 2.4%)	176.1 (3.7%; 3.6%)	175.9 (3.6%; 3.5%)
		[M+K] <sup>+</sup>	171.1 ± 0.3	171.1 ± 0.1	175.5 (2.5%; 1.5%)	-	175.5 (2.5%; 2.5%)
[M-H] <sup>-</sup>	164.0 ± 0.2	165.7 ± 0.1	168.2 (2.5%; 1.5%)	170.2 (3.6%; 2.6%)	171.4 (4.3%; 3.3%)		
[M+Cl] <sup>-</sup>	167.9 ± 0.2	169.2 ± 0.2	-	-	-		

## IMS of disaccharide isomers and their 6-phosphate derivatives



- $\Delta\text{CCS} < 1.5\%$  between  $^{\text{DT}}\text{CCS}_{\text{N}_2}$  and  $^{\text{TIMS}}\text{CCS}_{\text{N}_2}$  was observed for [M-H]<sup>-</sup>, [M+Cl]<sup>-</sup>, [M+Na]<sup>+</sup>, and [M+K]<sup>+</sup> ions.
- [M+K]<sup>+</sup> enabled better isomeric separation with ~2.5% difference between DTIMS and TIMS.
- Overall, the CCS values of trehalose are relatively larger than sucrose through this inter-platform IM profiling of disaccharide isomers.

## MALDI-TIMS-MSI of disaccharide isomers



**ACKNOWLEDGMENTS:** Funding was provided by the US Department of Energy (DOE), Office of Science, Office of Biological and Environmental Research (BER) Biomolecular Characterization and Imaging Science Program, and Bioimaging Research Approaches for the Bioeconomy & the Environment. A portion of this work was performed on a project award DOI: 10.46936/staf.proj.2023.60828/60008834 and DOI: 10.46936/staf.proj.2024.61572/60012825 from the Environmental Molecular Sciences Laboratory, a US DOE Office of Science User Facility sponsored by the BER program under Contract No. DE-AC05-76RL01830. The authors declare no competing financial interest.