Disclosure

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Application of Targeted Mass Spectrometry Imaging in the Pharmaceutical Industry

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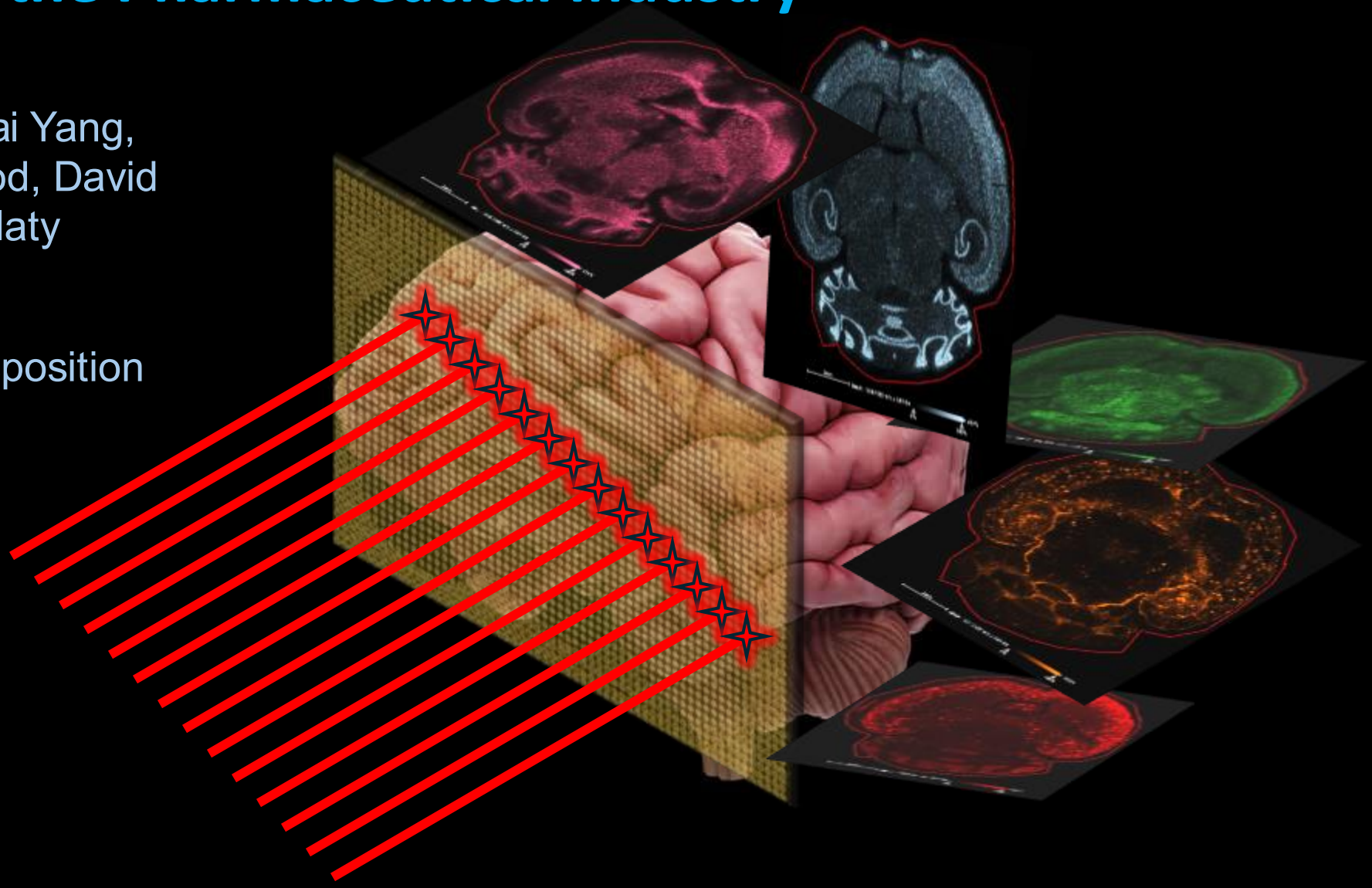
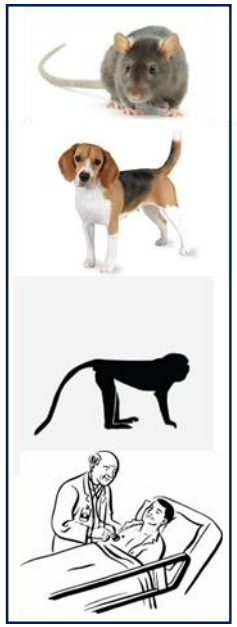
Drug Metabolism and Disposition





Matrices
(Blood, Urine, Feces etc.)

Tissues
(Liver, Kidney, Brain, Eye, Tumor)

LC-MS
(Matrices)
Homogenates

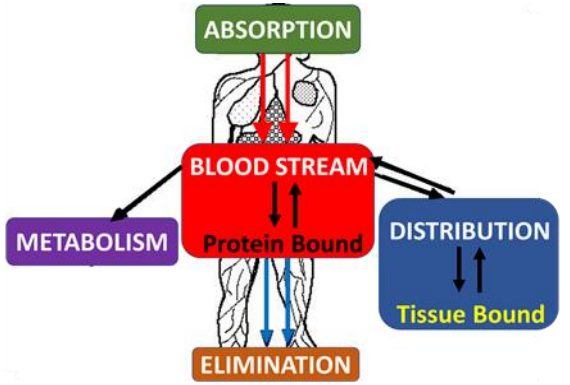
Distribution
Tissue Imaging
(MS, Optical, PET, etc

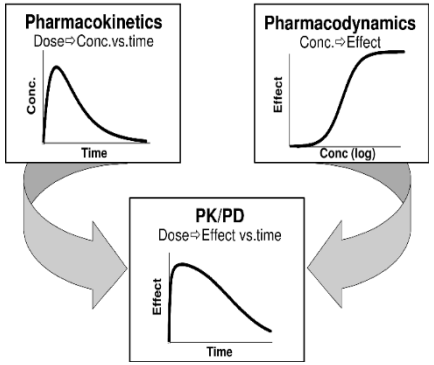
Pharmacokinetics
Fate of a Drug

Pharmacodynamics
Effect of Drug

Pharmaceutics
Science of dosage form design

Pharmacology
Drug Action

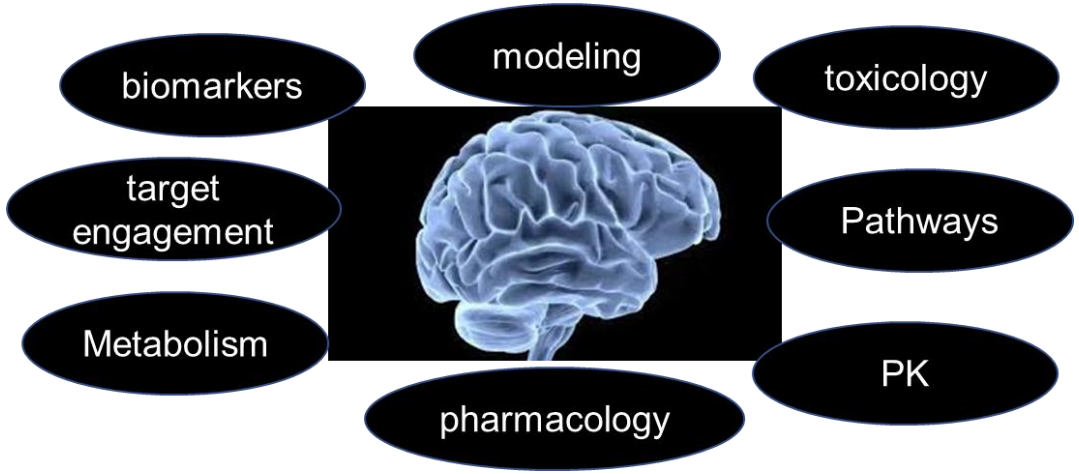




Tissue Imaging

- **Imaging provides distribution across tissue**
- **Detect changes in morphology and translate to disease state**
- **Follow physiological changes in tissue**

Quantitative Biodistribution of Drugs



- **Project teams need quantitative data**
- **If not quantitative, less valuable**
- **Pharmacology is a quantitative science**

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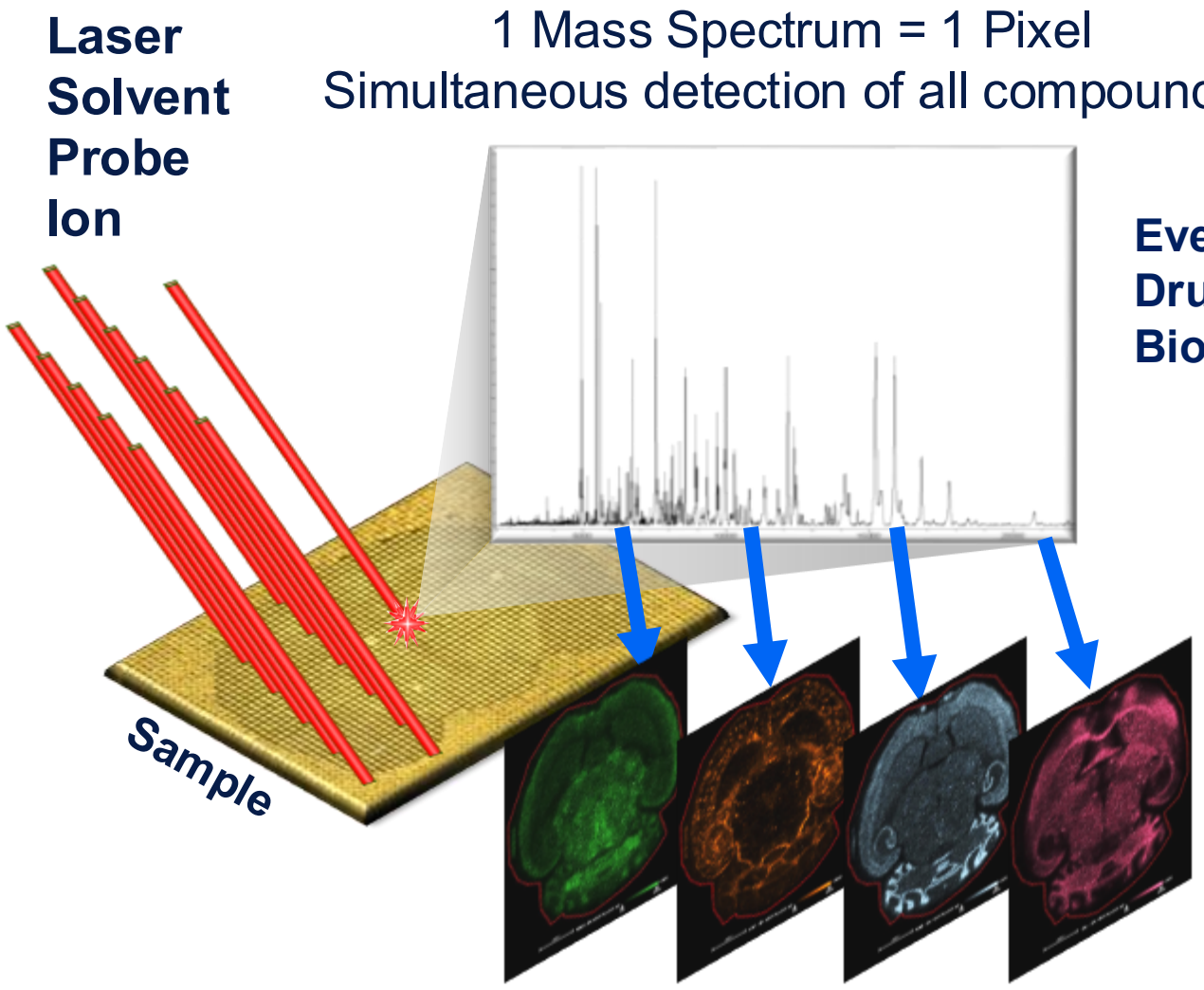
Mass Spectrometry Imaging



Everything on the Tissues:
Drug, Metabolites, Endogenous compounds, Biomarkers

- Drugs, metabolites, and endogenous compounds are desorbed from **discrete spots** in an ordered array.
- Each **pixel** is a **full mass scan** for **all compounds** ionized from the tissue spot.
- The abundances of individual ions are **independently plotted** per pixel to **visualize** their distribution as ion images.
- Each ion has its **own** image.
- **Compare** compound distribution to histology results
- **Correlate** with H&E, fluorescence, MRI, any other imaging technology

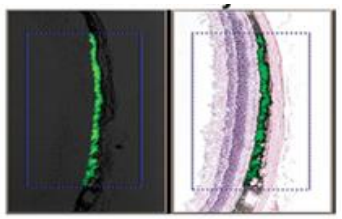
Utility of Distribution in Pharmaceutical Research

Molecular Histology

MSI combines high sensitivity and accuracy of MS for identification, with histology-like information (spatial distribution) within tissue sections

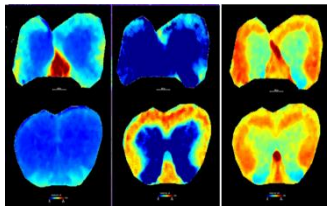
Target Identification → **Lead Generation Optimization** → **Candidate Selection and Development** → **Pre-clinical Development** → **Clinical Development**

Target Engagement



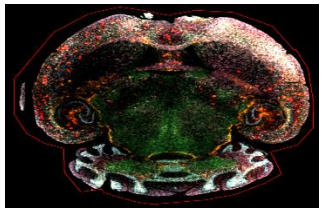
- Reaching target
- Target Occupancy
- Time at target
- Efficacy
- Compound selection
- Formula selection

Biomarker Identification



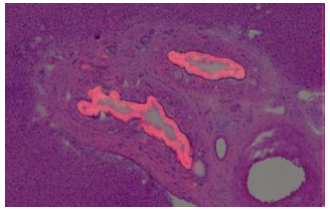
- Markers of efficacy
- Explore signaling mechanisms
- Understand pathology to discover therapeutic targets

Multi-modal Integration



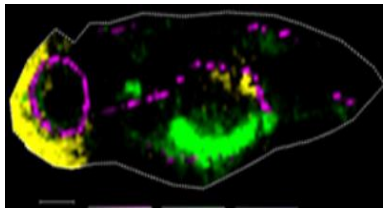
- Relate morphology to distribution
- Hepatotoxicity
- CNS penetration
- Tumor distribution
- PK/PD relationship

Toxicity Support



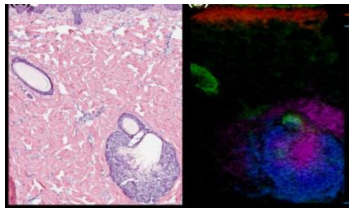
- Safety
- ID molecules responsible for toxicity

Tissue Distribution



- Target distribution
- Metabolism
- Clearance
- Tissue PK/PD
- Exposure studies
- Drug accumulation

Clinical



- PK/PD in human tissues
- Dose response
- Efficacy
- Target

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Characteristics of MS Imaging

Advantages

- Spatially resolved molecular information across tissue
- Label-free analysis
- MSI can detect a wide range of molecules with good sensitivity and specificity
- Simultaneously detect and analyze thousands compounds
- Combined with (MS/MS), can provide structural information

Disadvantages

- Generates large, complex datasets that require advanced analysis techniques.
- Requires meticulous sample preparation, which can affect data quality
- Quantification challenges
- Instrumentation cost and complexity

Mass spectrometry imaging has proven critical in the pharmaceutical industry, but its broad utility requires addressing key challenges of sensitivity and cost

Can be facilitated by Targeted Imaging

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Untargeted vs Targeted MSI

Untargeted Mass Spectrometry Imaging

Pros:

- **Spatial mapping** broad range of molecules, without prior knowledge
- Ideal for exploratory studies (new or unexpected compounds)

Cons:

- Generates large, complex datasets
- Generally semi-quantitative
- **Lower Sensitivity**

Applications: Commonly used in studies focused on identifying/discovering molecules across the tissues: **understanding of** disease signaling mechanisms, biomarker **discovery, metabolism** across tissues.

Targeted Mass Spectrometry Imaging

Pros:

- **High Sensitivity and Specificity**
- Allows for more accurate quantification.
- **Simplified Data Analysis**

Cons:

- Only predetermined molecules are analyzed
- **Lack of Exploratory Potential**

Applications: Best suited for applications requiring detailed investigation of known compounds, target engagement, and monitoring specific drug/metabolite distributions or biomarkers in tissues.

Currently, a **targeted MALDI platform is NOT commercially available** that combines high spatial resolution, highly robust workflow, and user-friendly experience

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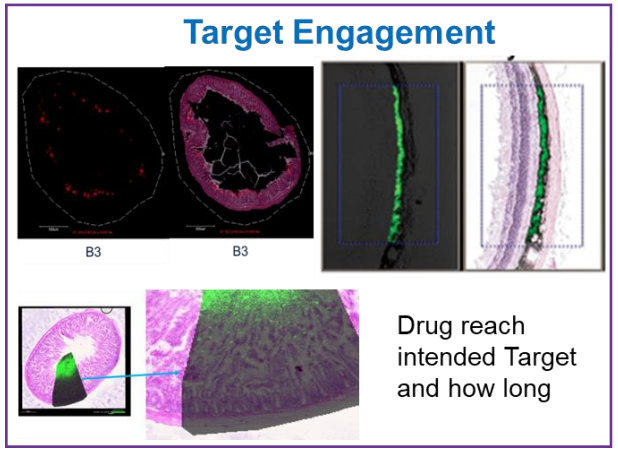
Collaboration with Agilent for Targeted Imaging using MALDI

Focus on improving sensitivity

Drug Efficacy (Target Engagement)
Biomarker monitoring
Tissue PK/PD
Drug Accumulation

Compounds

Low dosed compounds
ADC moieties (payload)
Quantitation



Agilent

- 6495D state of the art QqQ
- Provide strategies to obtain best sensitivity and data quality
- Provide support with data processing
- Provide support for hardware modifications

Mass Tech

- Provide AP-MALDI Source
- Ability to modify inlet system

AbbVie

- Provide real world samples and applications
- Determine scientific gaps and robustness
- Provide ideas for enhancing technology

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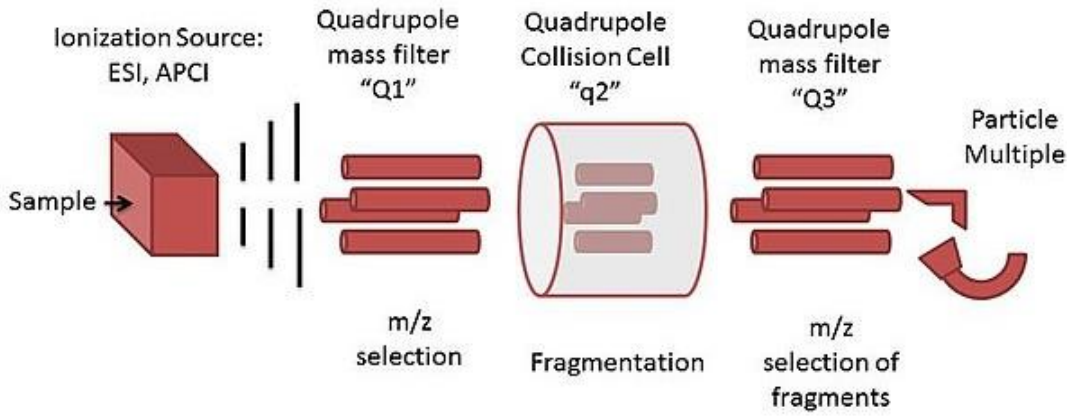
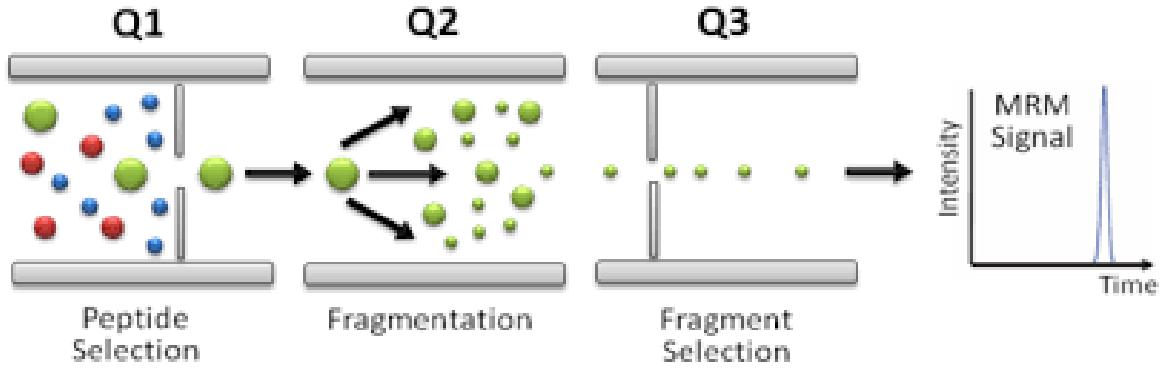
Triple Quadrupole MS Imaging

Agilent 6495D

MassTech AP-MALDI UHR

QqQ Schematic





- ✓ Significant increase in sensitivity
- ✓ Targeted imaging (only looking for known molecules)
- ✓ Useful for low tissue concentrations

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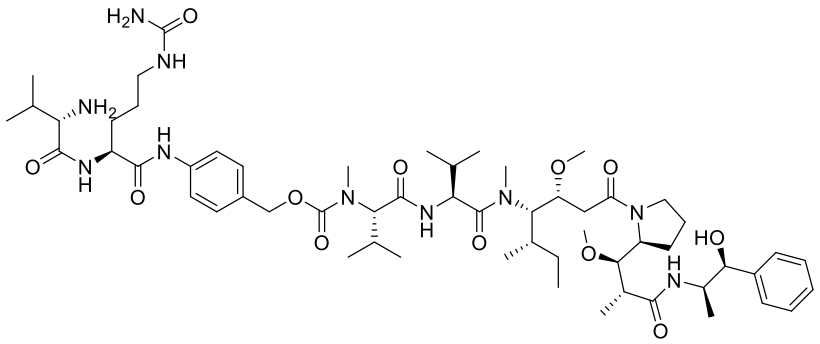
Preliminary: Sensitivity Increase with Agilent Targeted MSI

Compared to broad spectrum detection

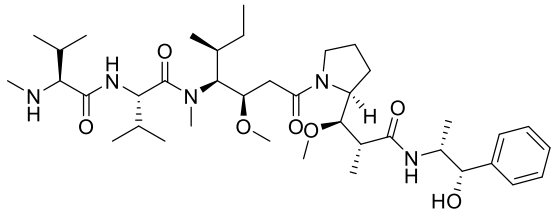
Payload MMAE-linker



Exact Mass: 1122.70527

170-fold better LOD
56-fold better LOQ

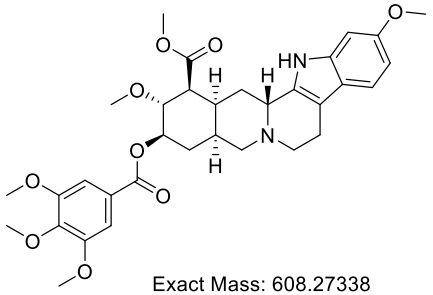
Payload MMAE



Exact Mass: 717.50405

16-fold better LOD
7-fold better LOQ

Reserpine



Exact Mass: 608.27338

1-fold better LOD
1-fold better LOQ

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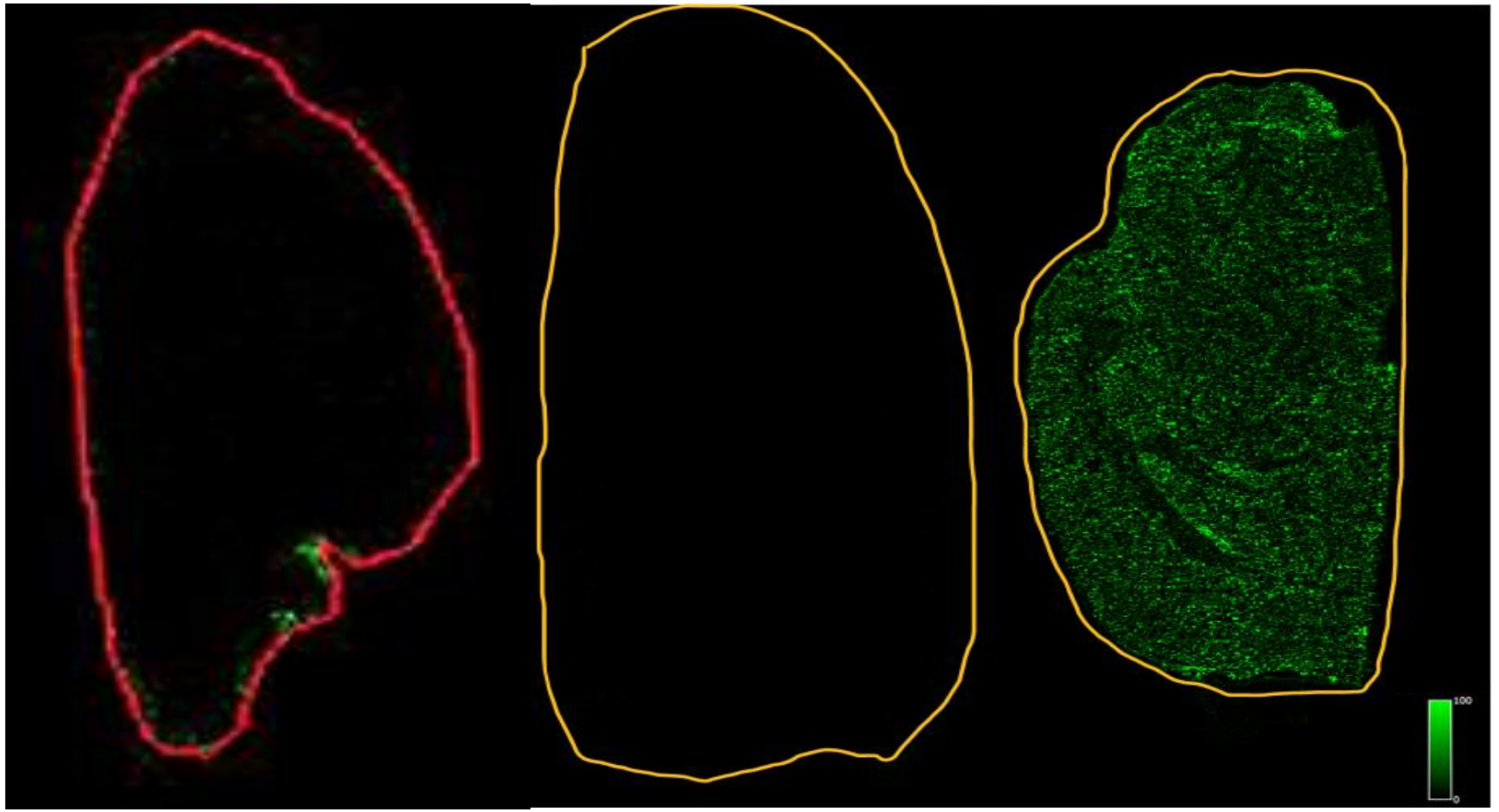
Sensitivity of Targeted Imaging vs. Scanning Mode

Visualizing low-dose drug compound



timsTOF Flex
Untargeted
Dosed

QqQ
Targeted
Vehicle Control

QqQ
Targeted
Dosed

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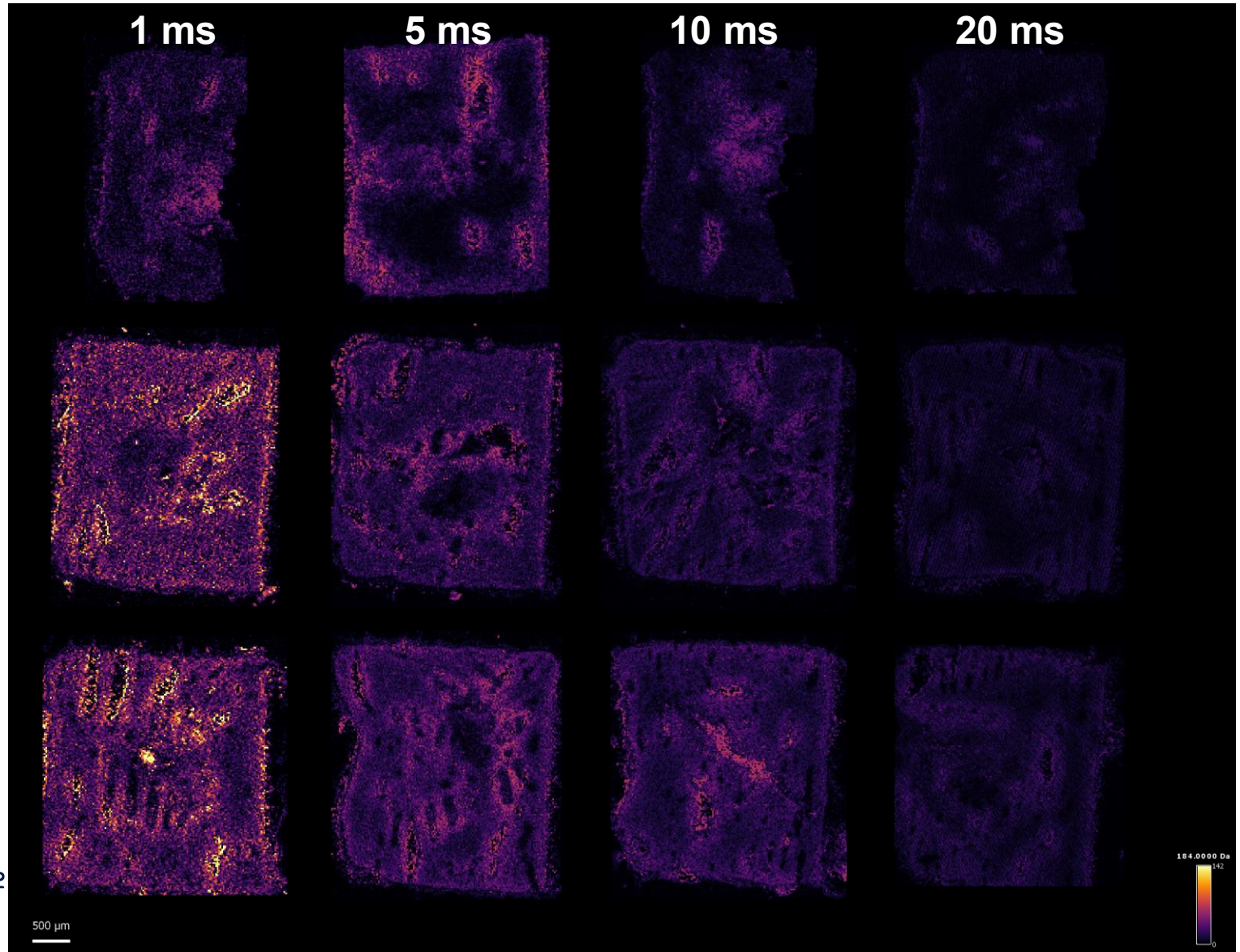
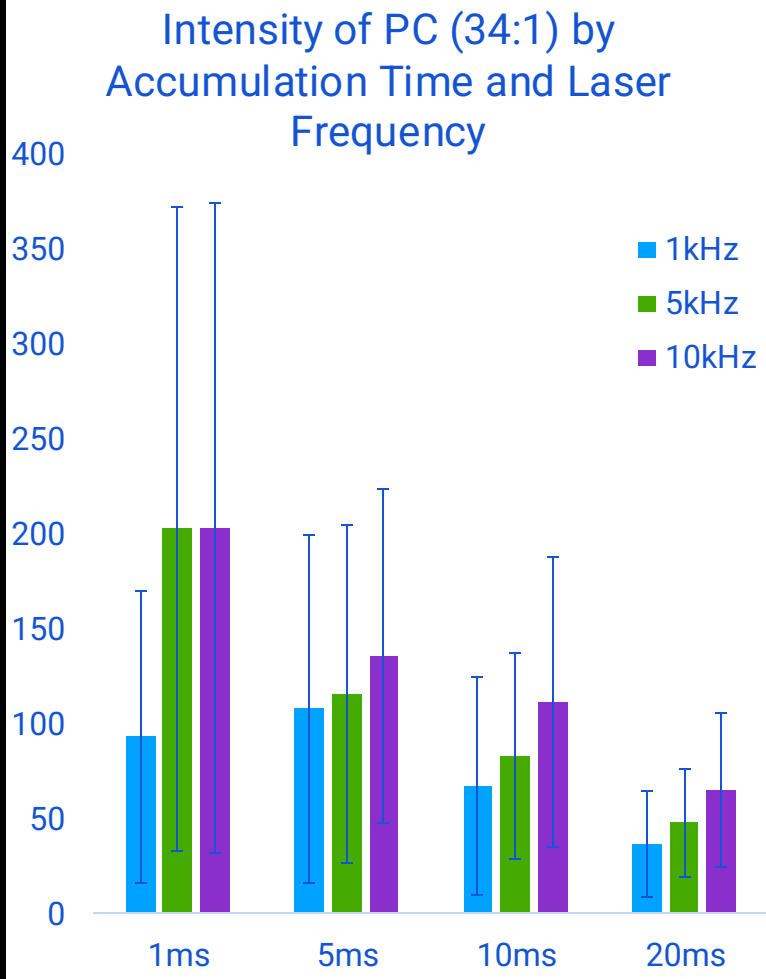
Running the MassTech source: Adjusting Frequency and Accumulation Time for an Endogenous Lipid (PC 34:1)





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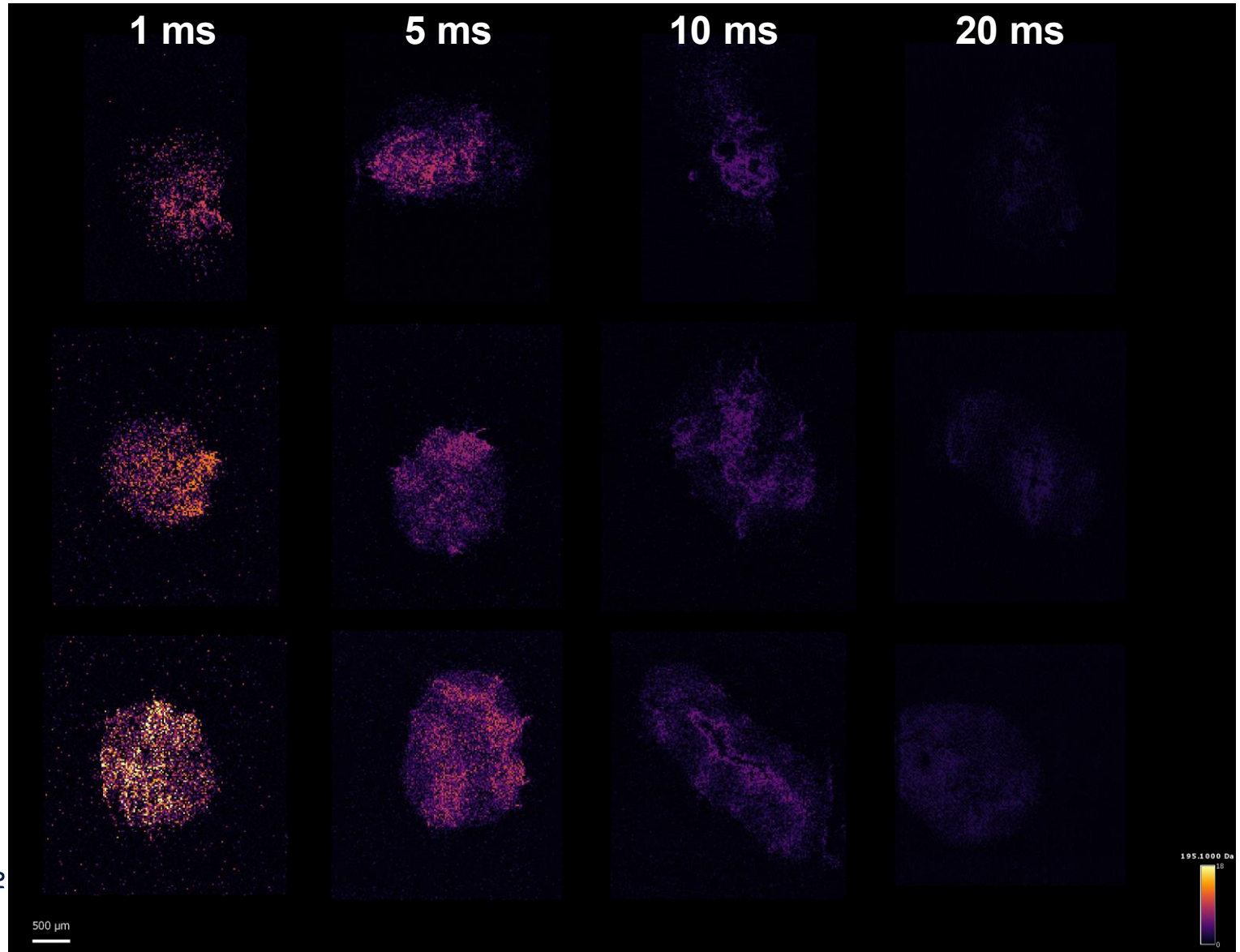
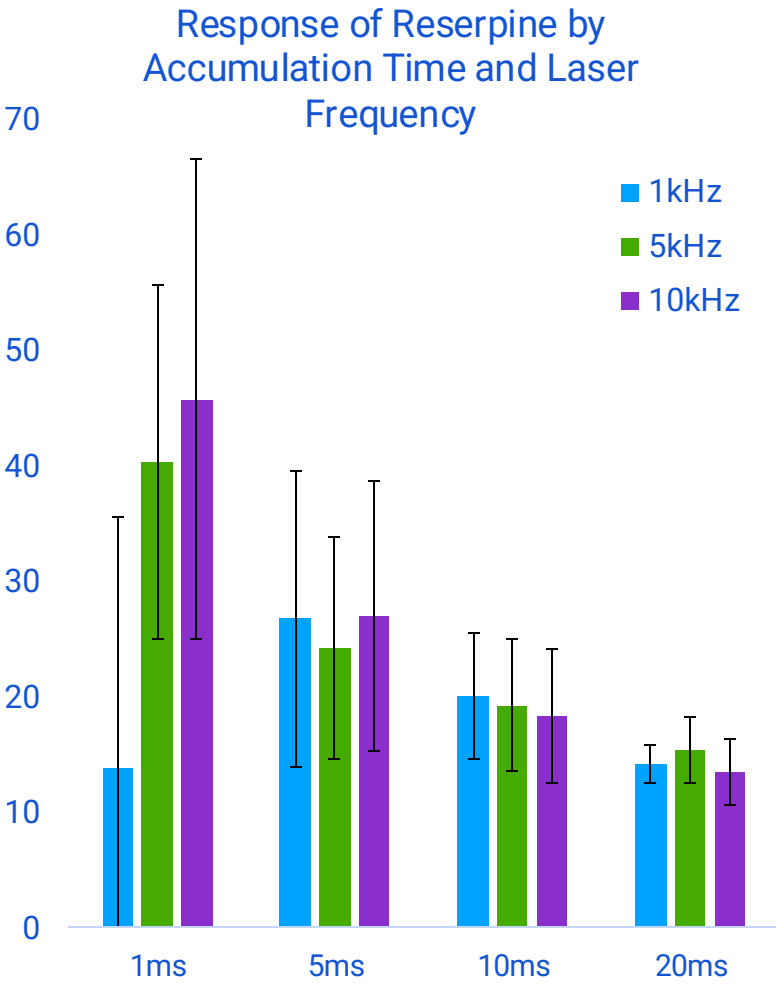
Running the MassTech source: Adjusting Frequency and Accumulation Time for a Spotted Analyte (Reserpine)





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Conclusions and Future Work

- **Targeted MS imaging** is increasingly important in supporting drug discovery, development, and clinical trials within the pharmaceutical industry.
- There is a **growing need** for a high-throughput targeted MS imaging platform that offers high spatial resolution for analyzing drug compounds, metabolites, disease markers, and treatment markers.
- Initial experiments demonstrate the **significant potential of combining AP-MALDI with QqQ** to serve as a targeted MS imaging platform and address existing gaps.
- Continue to develop QqQ methods and data analysis pipelines (file conversion) for more compounds and applications
- Determine sensitivity increases across broad compound classes
- Implement rapid MRM scanning workflow

Acknowledgements

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Sheher Mohsin – Applications Chemist
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