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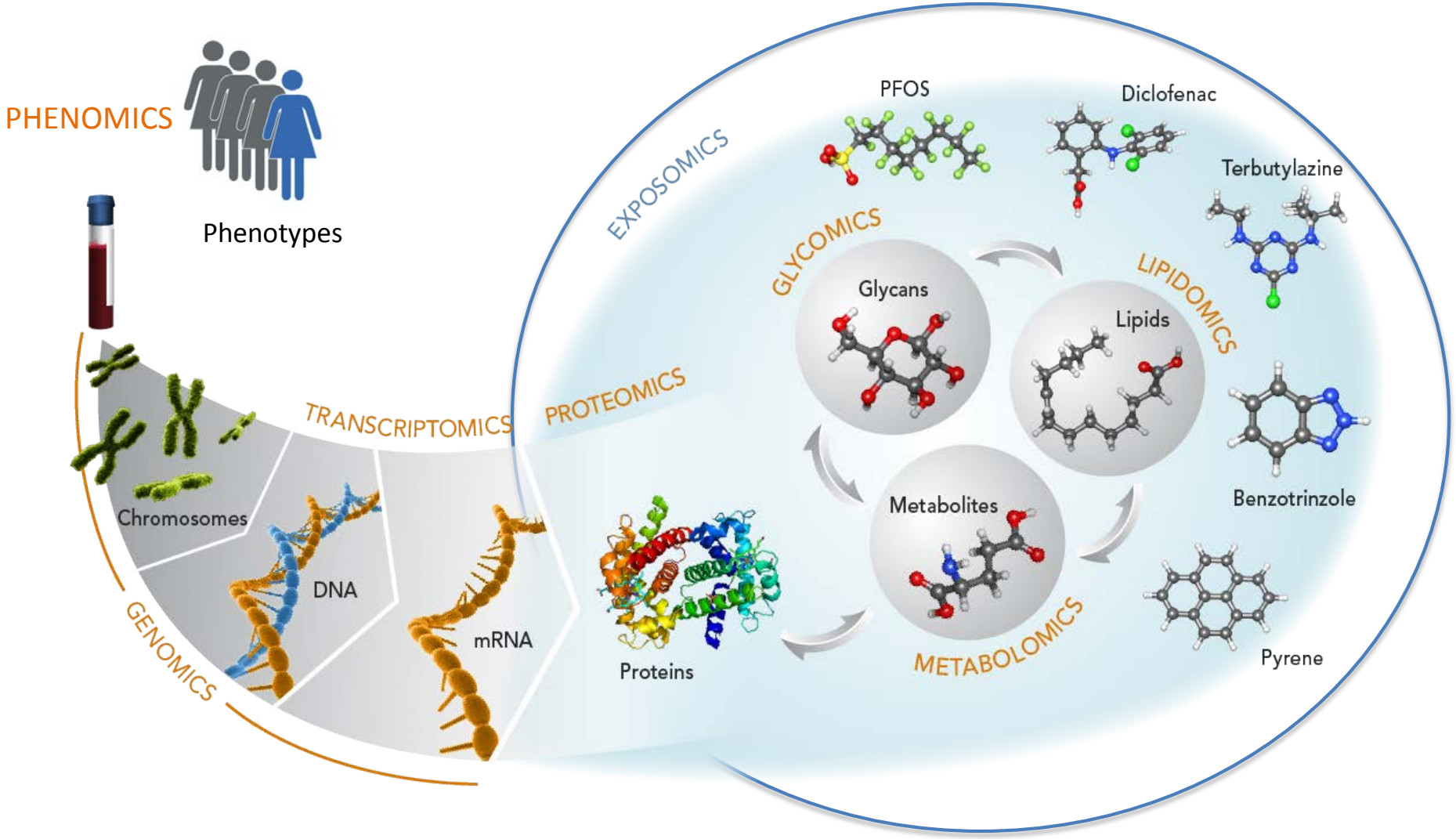
Building a Library with >1000 Ion Mobility Collision Cross Sections for Ultrafast Small Molecule Analyses

Xueyun Zheng, Noor A. Aly, Vanessa L. Paurus, Yuxuan Zhou, Daniel J. Orton, Richard D. Smith and Erin S. Baker

Biological Sciences Division, Pacific Northwest National Laboratory

65th ASMS Conference on Mass Spectrometry and Allied Topics, 2017

Multi-omic Future



MS-based Measurements

Major challenges for small molecule measurements

1. Wide concentration range (fM-mM) requires measurements with high dynamic range and sensitivity
2. Biological changes are best understood with both endogenous metabolites and xenobiotics analyzed
3. Untargeted measurements covering thousands of small molecules are desired for time course studies and large clinical/environmental studies

Major challenges for small molecule measurements

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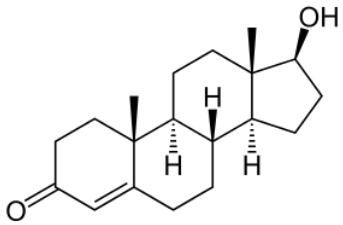
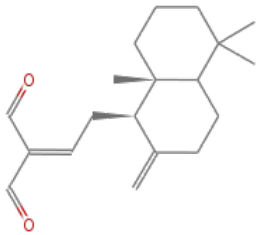
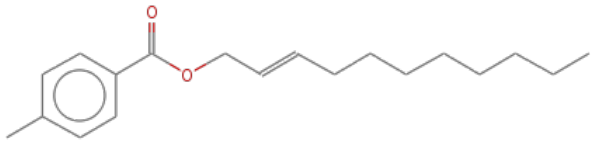
2. Biological changes are best understood with both endogenous metabolites and xenobiotics analyzed
3. Untargeted measurements covering thousands of small molecules are desired for time course studies and large clinical/environmental studies

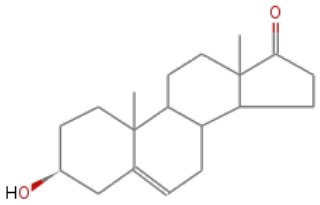
4. Diversity of chemical structures and isomer forms so distinguishing them with conventional MS or chromatographic approaches can be difficult or impossible



Testosterone
Exact mass = 288.2089188



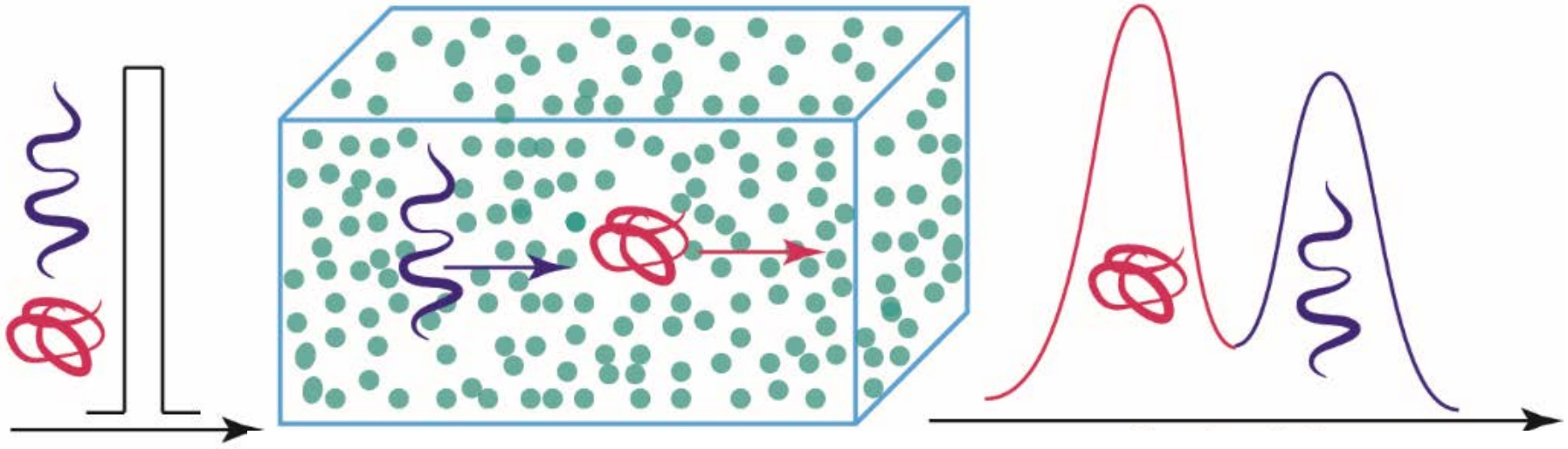




In the NIST database there are
18 different options with exact mass = 288.2089188

Ion mobility concept

Conformation Separation

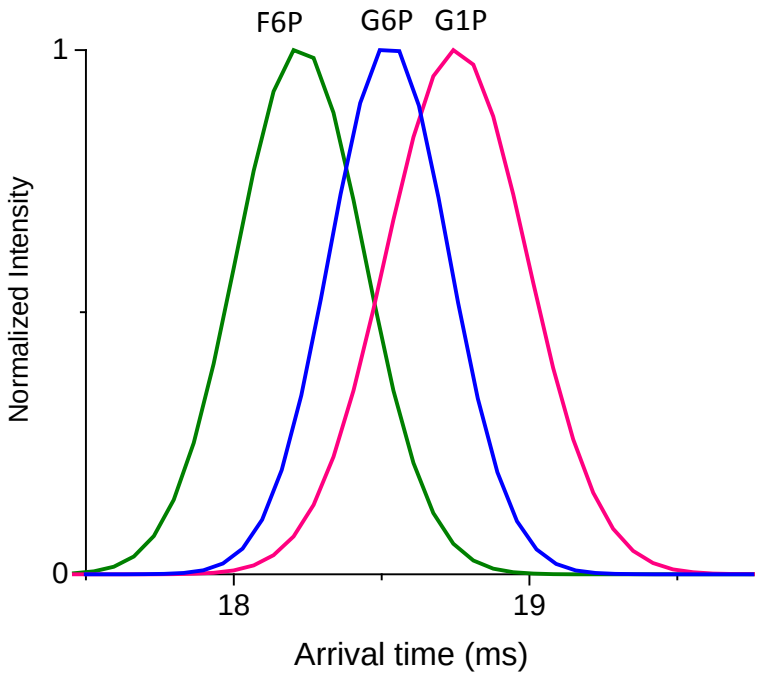


Isomeric separation of endogenous metabolites

D-Fructose-6-phosphate
(F6P)

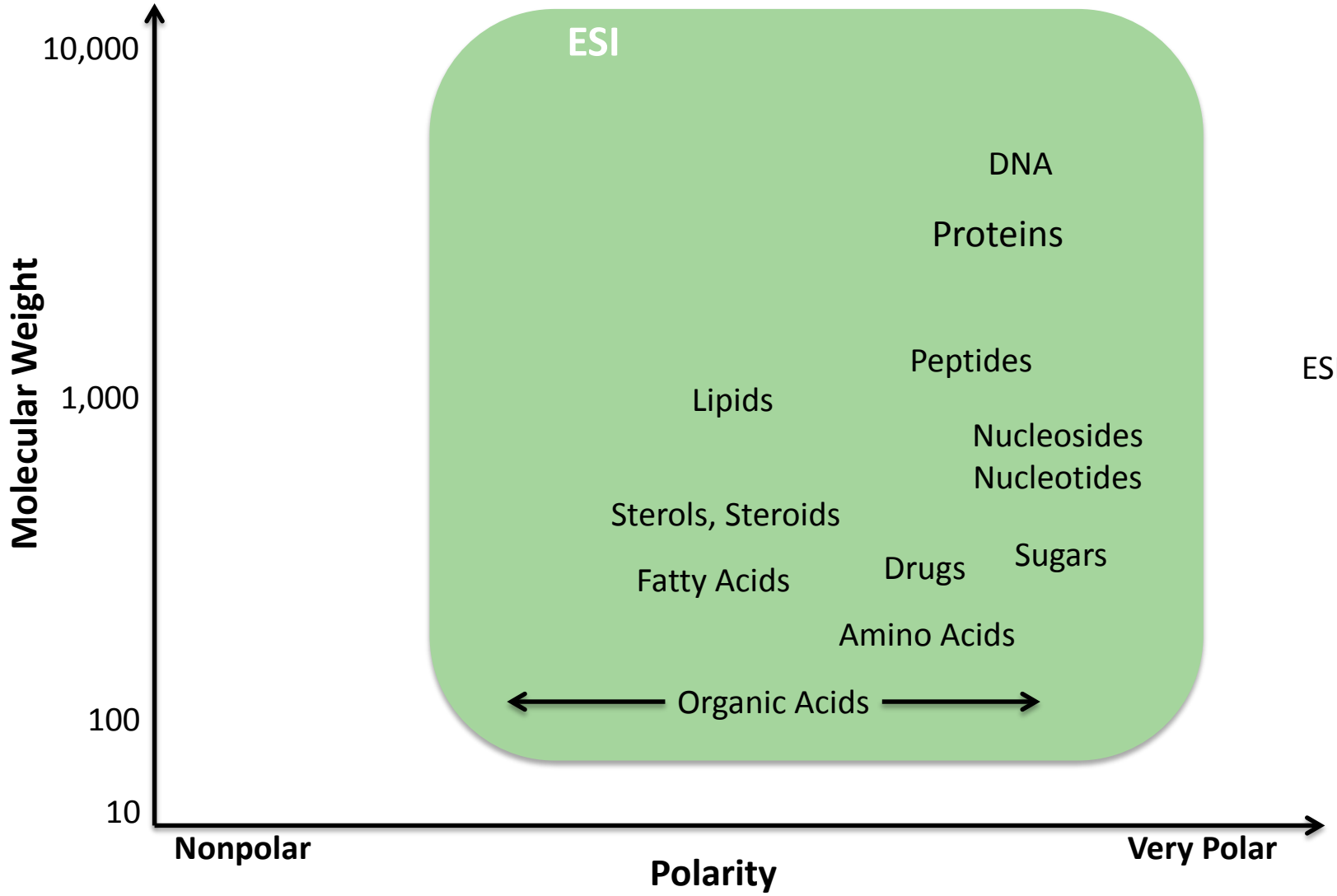
D-Glucose-6-phosphate
(G6P)

α-D-Glucose-1-phosphate
(G1P)



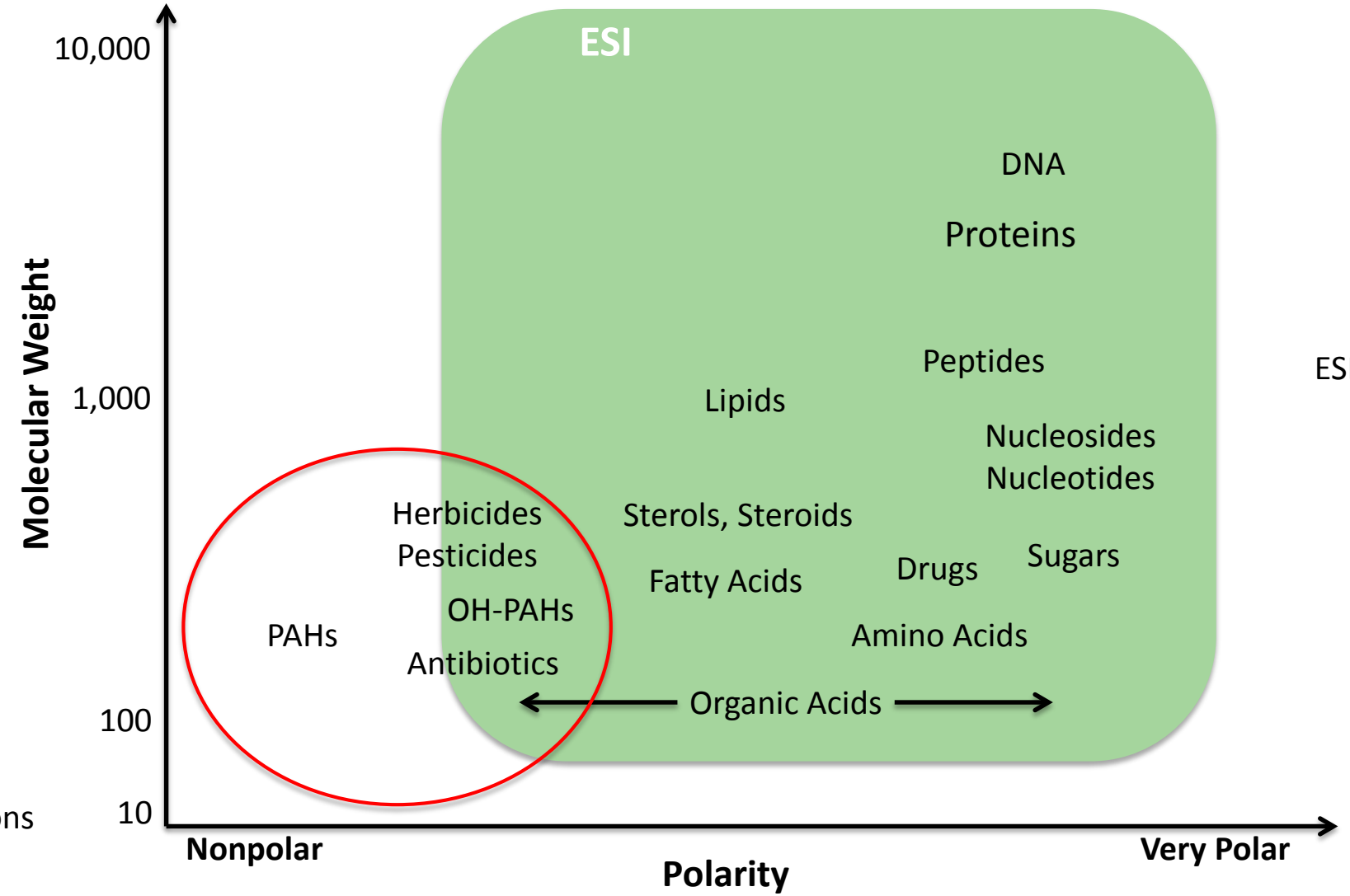
Sodiated form

Using different ionization methods for different molecules



ESI: Electrospray ionization

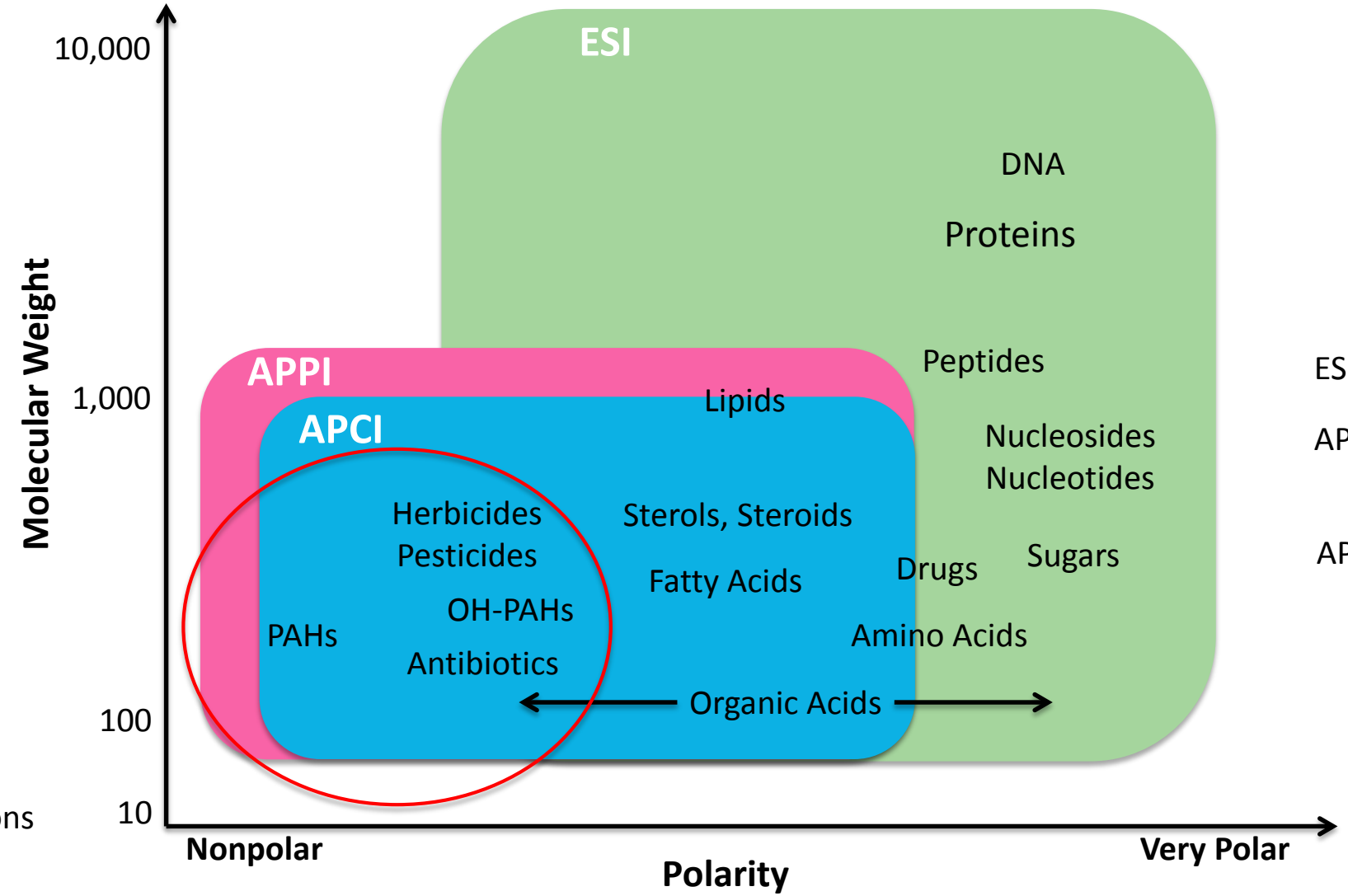
Using different ionization methods for different molecules



ESI: Electrospray ionization

PAHs: Polycyclic Aromatic Hydrocarbons

Using different ionization methods for different molecules



ESI: Electrospray ionization

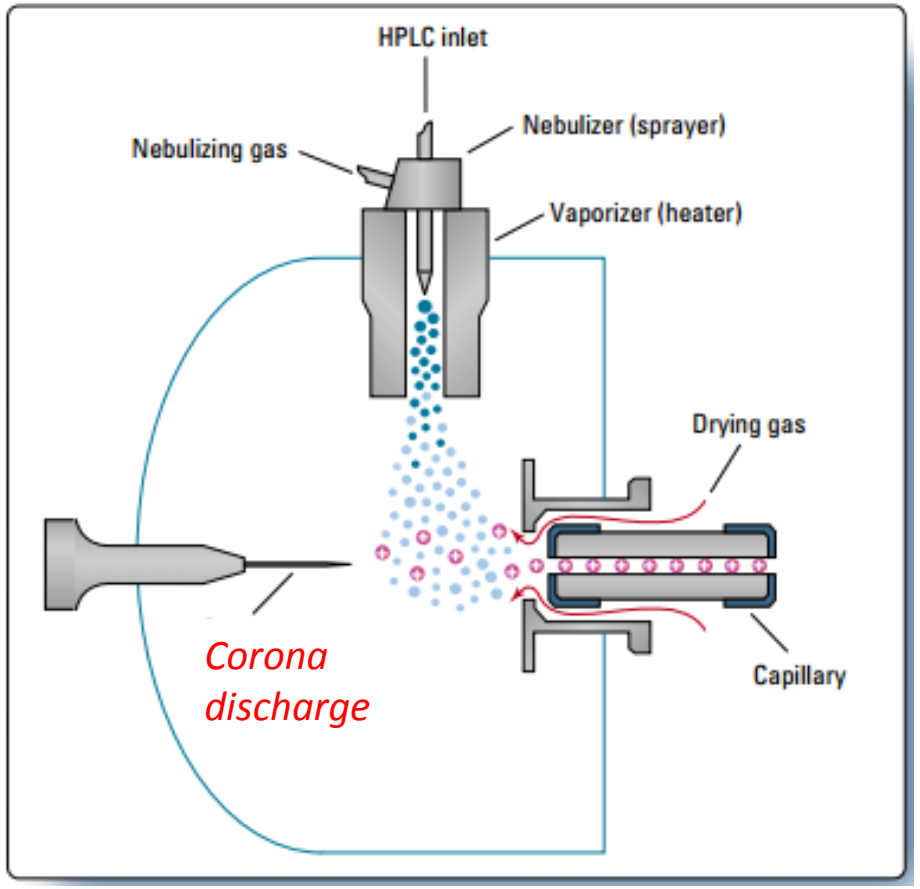
APCI: Atmospheric pressure chemical ionization

APPI: Atmospheric pressure photoionization

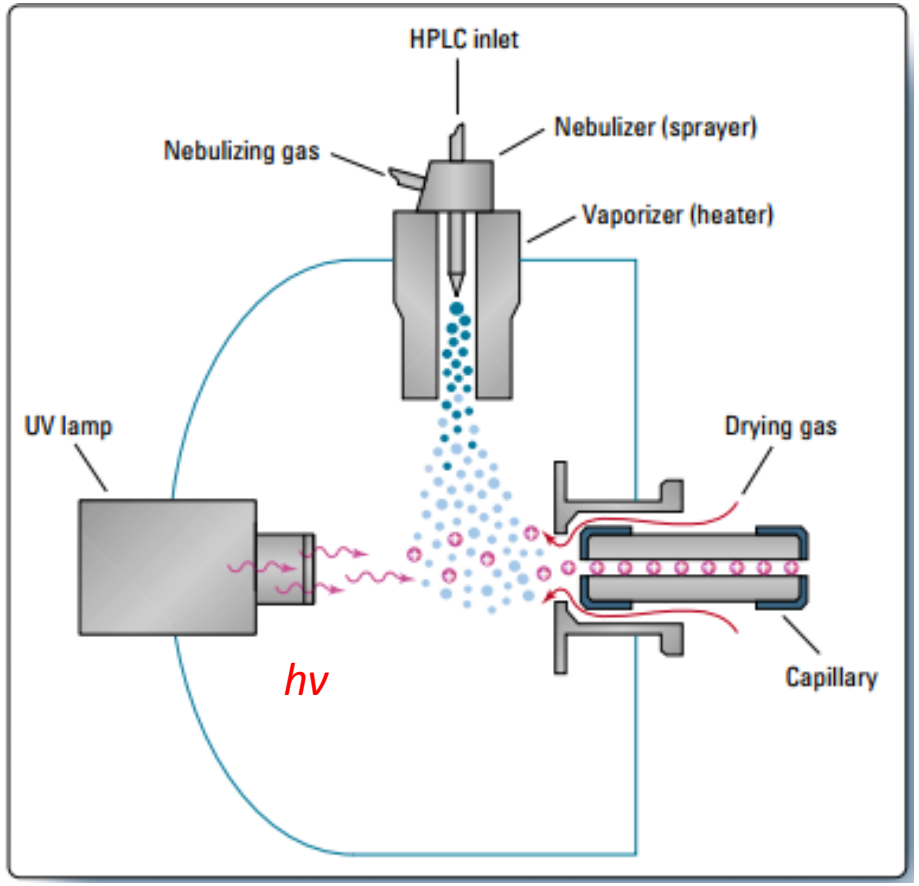
PAHs: Polycyclic Aromatic Hydrocarbons

Using different ionization methods for different molecules

APCI
Atmospheric pressure chemical ionization

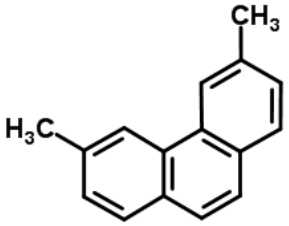


APPI
Atmospheric pressure photoionization

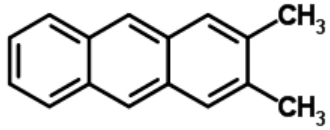


APPI and/or APCI ionization for xenobiotic analyses

Polycyclic aromatic hydrocarbons (PAHs) were detected in the radical and/or protonated forms using APCI and APPI.

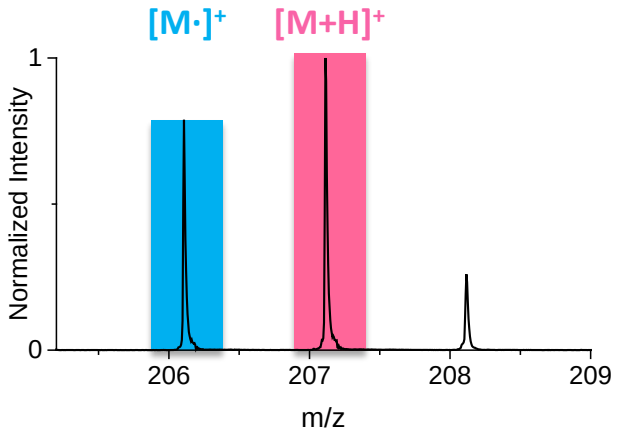


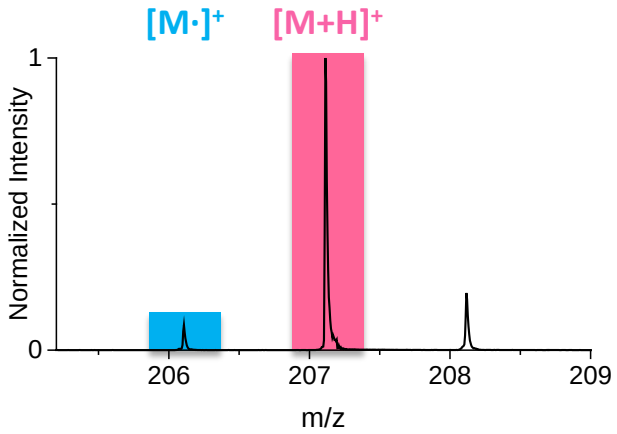
3, 6-Dimethylphenanthrene



2, 3-Dimethylanthracene

APCI





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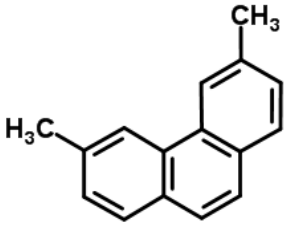

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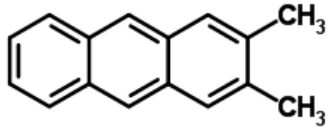

2, 3-Dimethylanthracene

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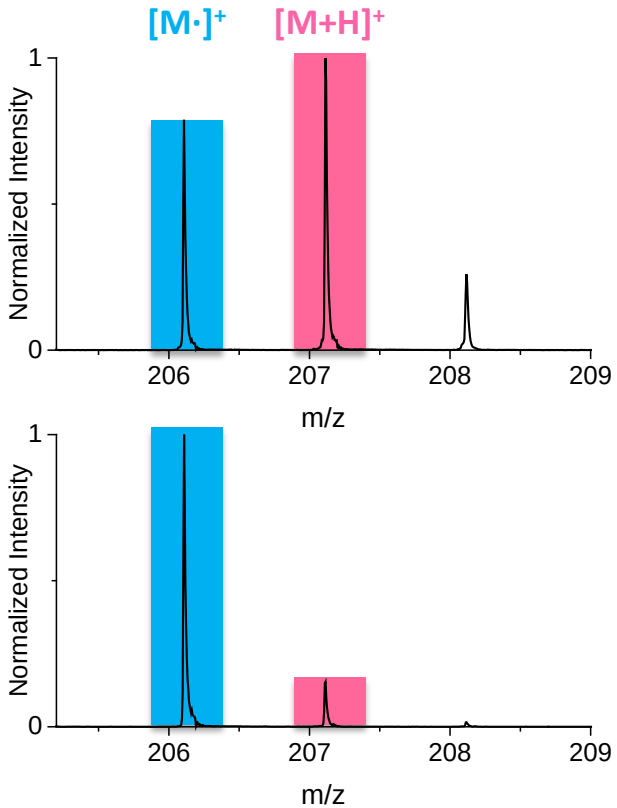
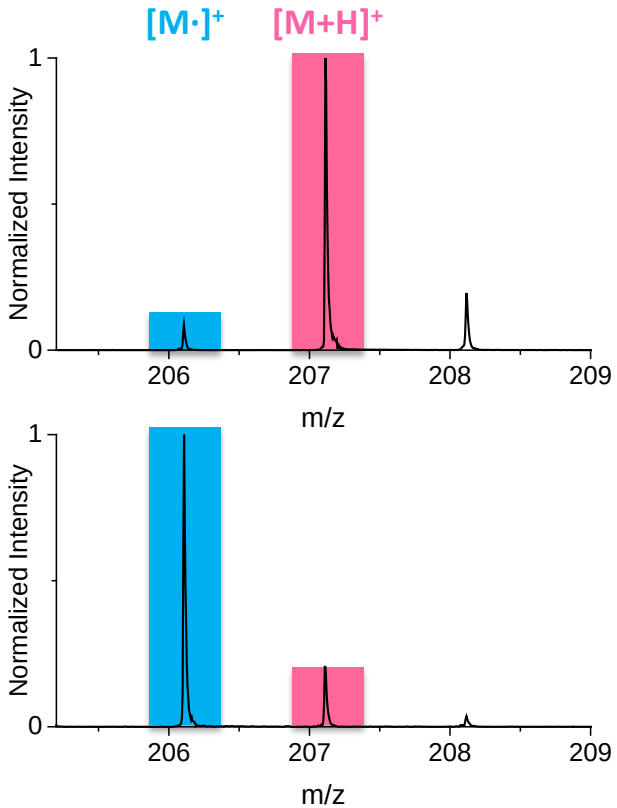
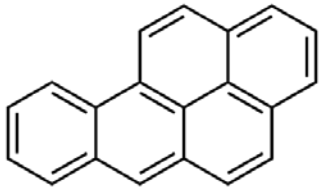


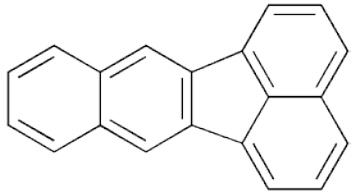


Isomeric xenobiotic separations

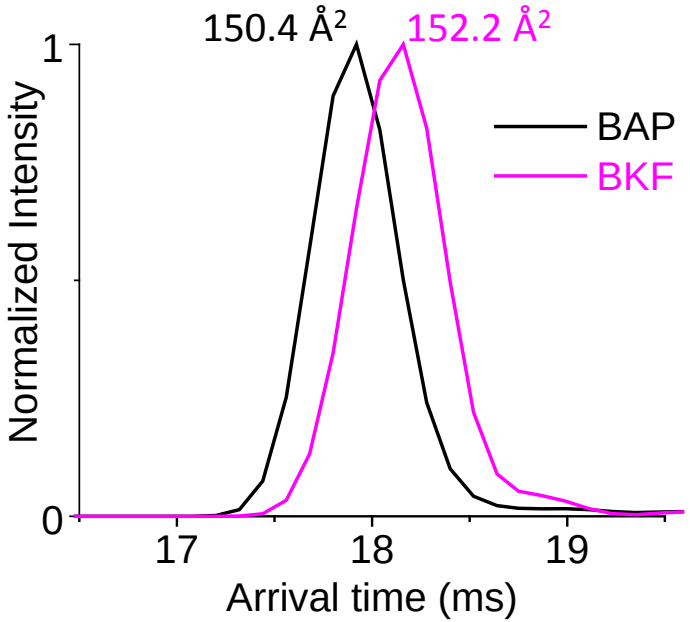
Polycyclic aromatic hydrocarbons (PAHs)



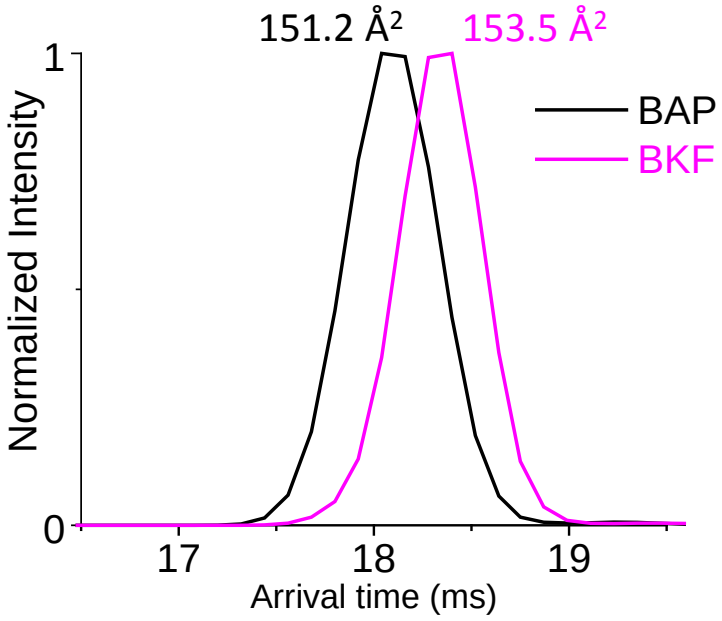
BAP: Benzo(a)Pyrene



BKF: Benzo(k)fluoranthene

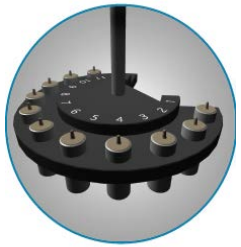


Radical form $[M\cdot]^+$



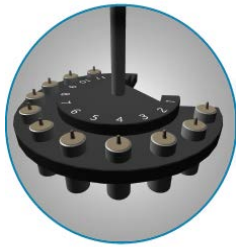
Protonated form $[M + H]^+$

Automated SPE system enabling ultrafast analyses

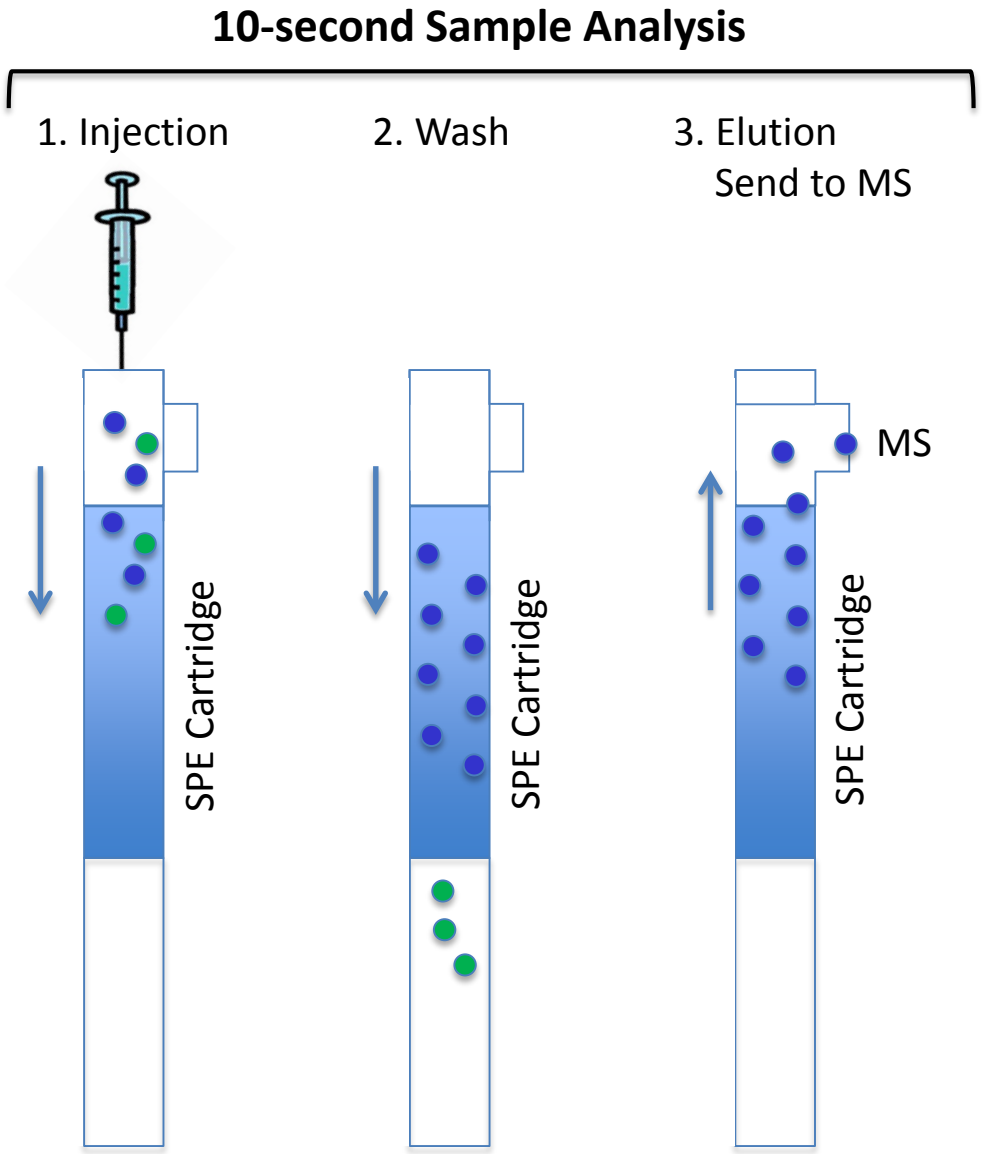


Rapidfire SPE system

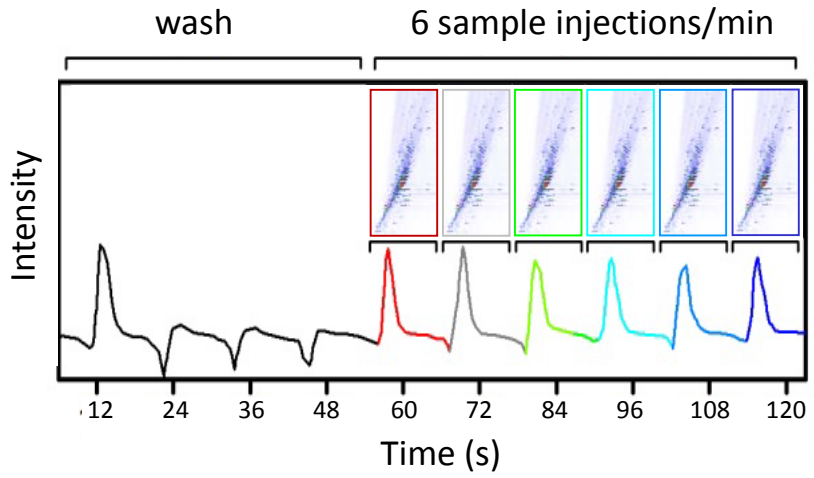
Automated SPE system enabling ultrafast analyses

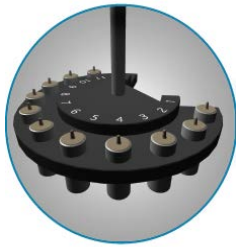


Rapidfire SPE system



Automated SPE system enabling ultrafast analyses





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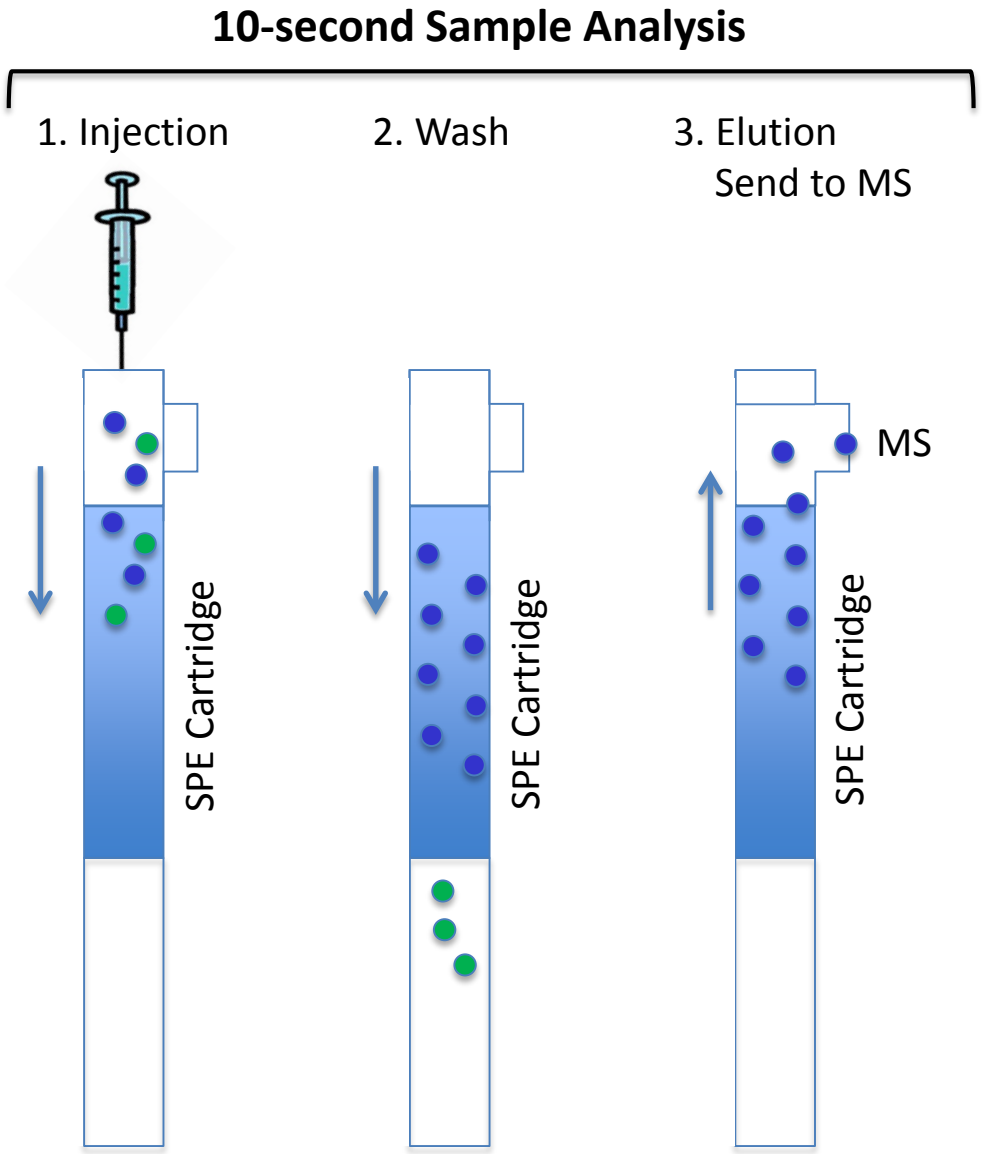
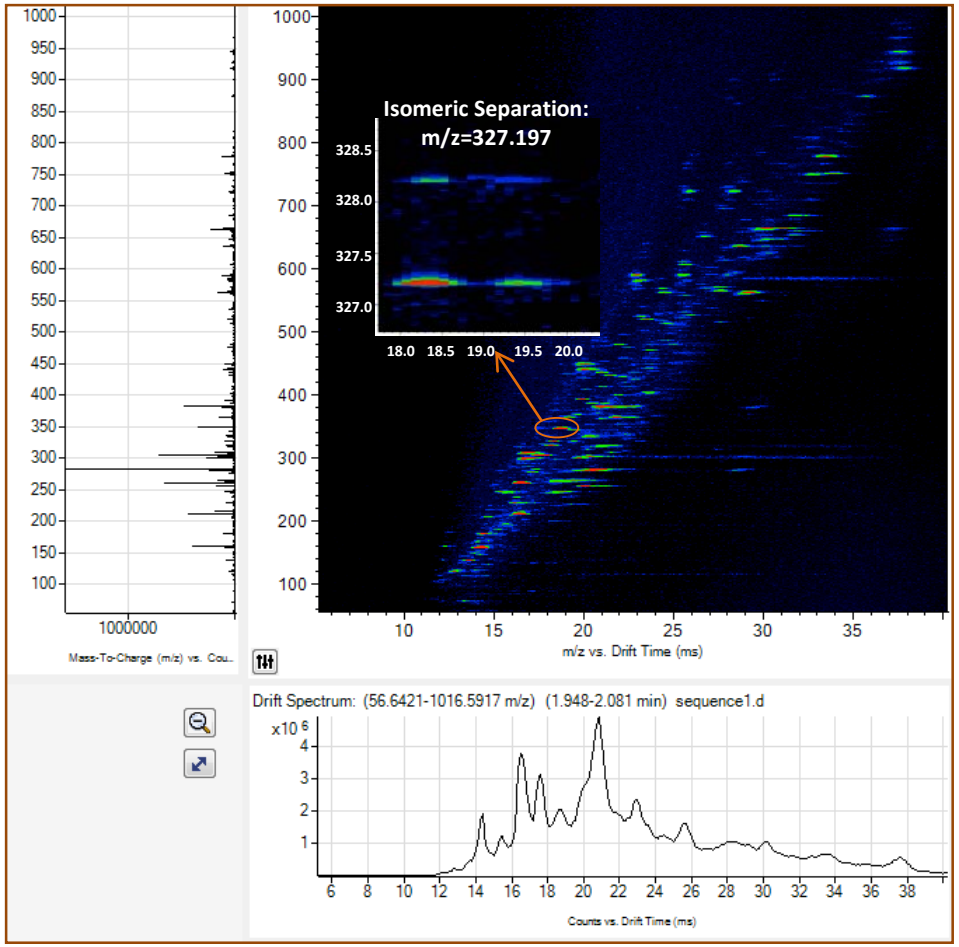


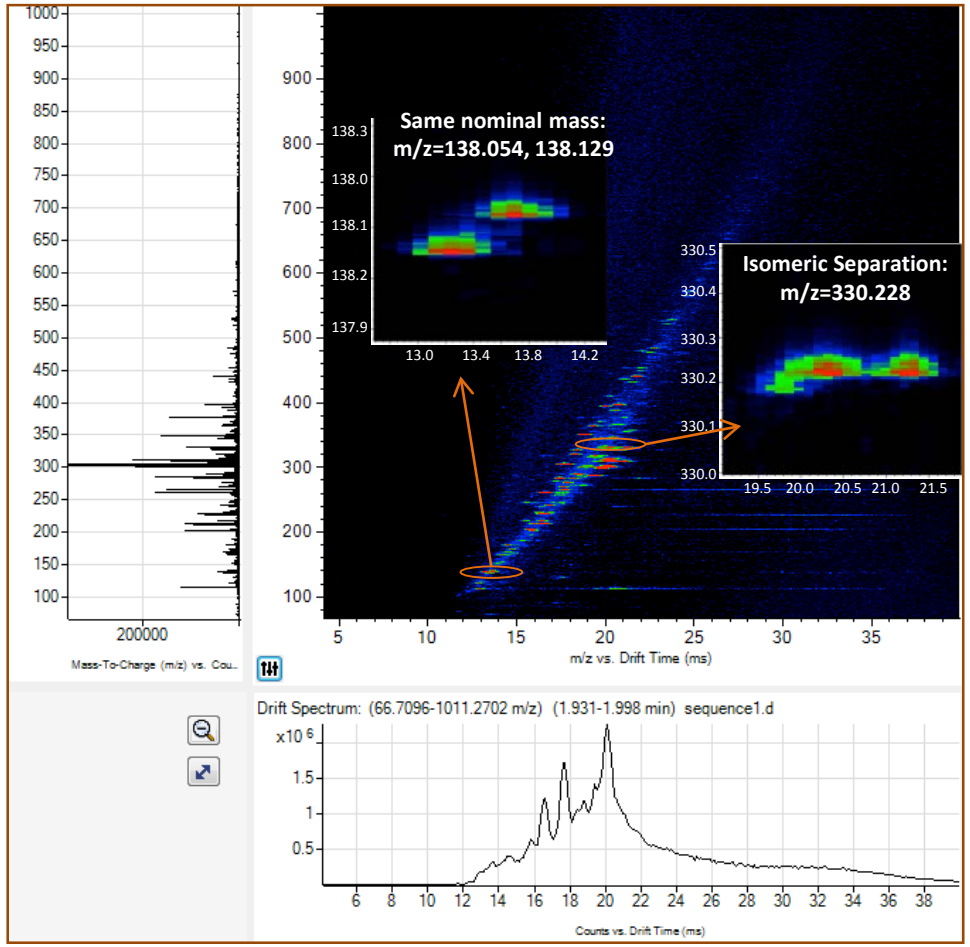
SPE-IMS-MS analyses of biological samples

Metabolites extracted from mouse plasma



~1400 features with S/N > 5

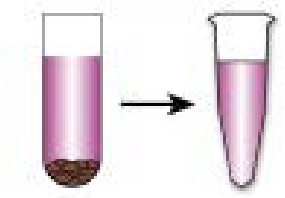
Metabolites extracted from human urine



~1000 features with S/N > 5

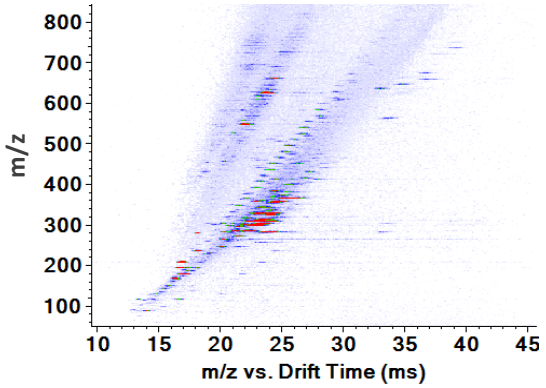
Metabolomics and xenobiotics pipeline

Extraction



Methanol/Chloroform Extraction
(100 samples/day)

Instrumental Analysis



SPE-IMS-MS

(8200 samples/day)

Data Processing & Analysis

ID	Mass	CCS	Intensity
----	----	----	----
----	----	----	----
----	----	----	----
----	----	----	----
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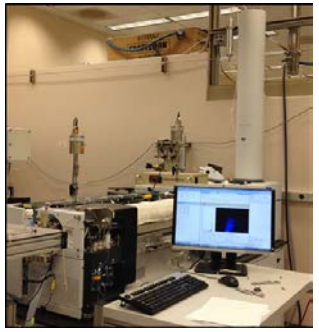
Database Matching &
False Discovery Assessment
(? day)

Direct drift tube IMS CCS measurement workflow

Metabolite Standards

7-voltage IMS measurements



Direct drift tube IMS CCS measurement workflow

Metabolite Standards

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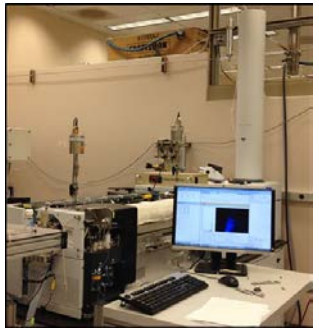
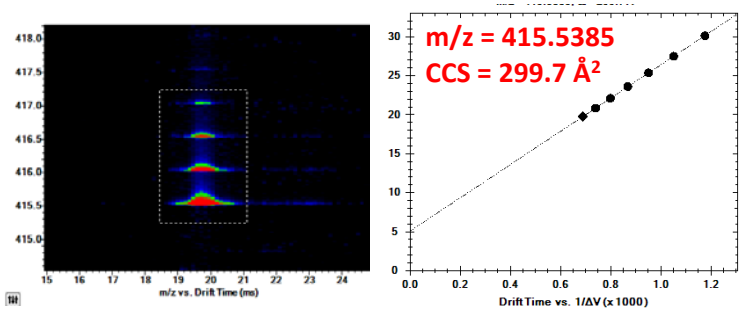


7-voltage CCS Calculations

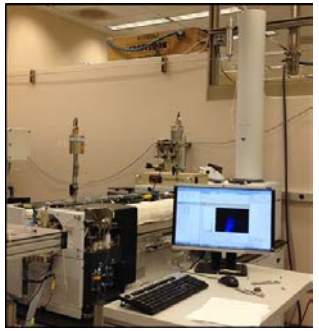


$$\mathrm{CCS} = 1.3\left(\frac{q^2E^2T}{\mu\, k_B p^2 N^2 l^2}\right)^{1/2}(t_A - t_0)$$

Direct drift tube IMS CCS measurement workflow

Metabolite Standards

7-voltage IMS measurements

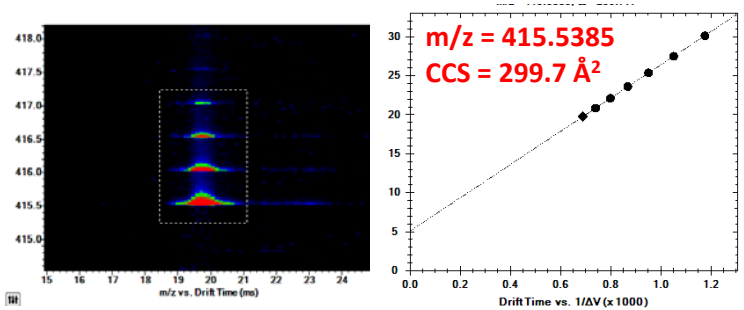


CCS Database

ID	Mass	CCS1	CCS2	CCS3	CCS_{Avg}	RSD%
----	----	----	----	----	----	----
----	----	----	----	----	----	----
----	----	----	----	----	----	----
----	----	----	----	----	----	----
----	----	----	----	----	----	----

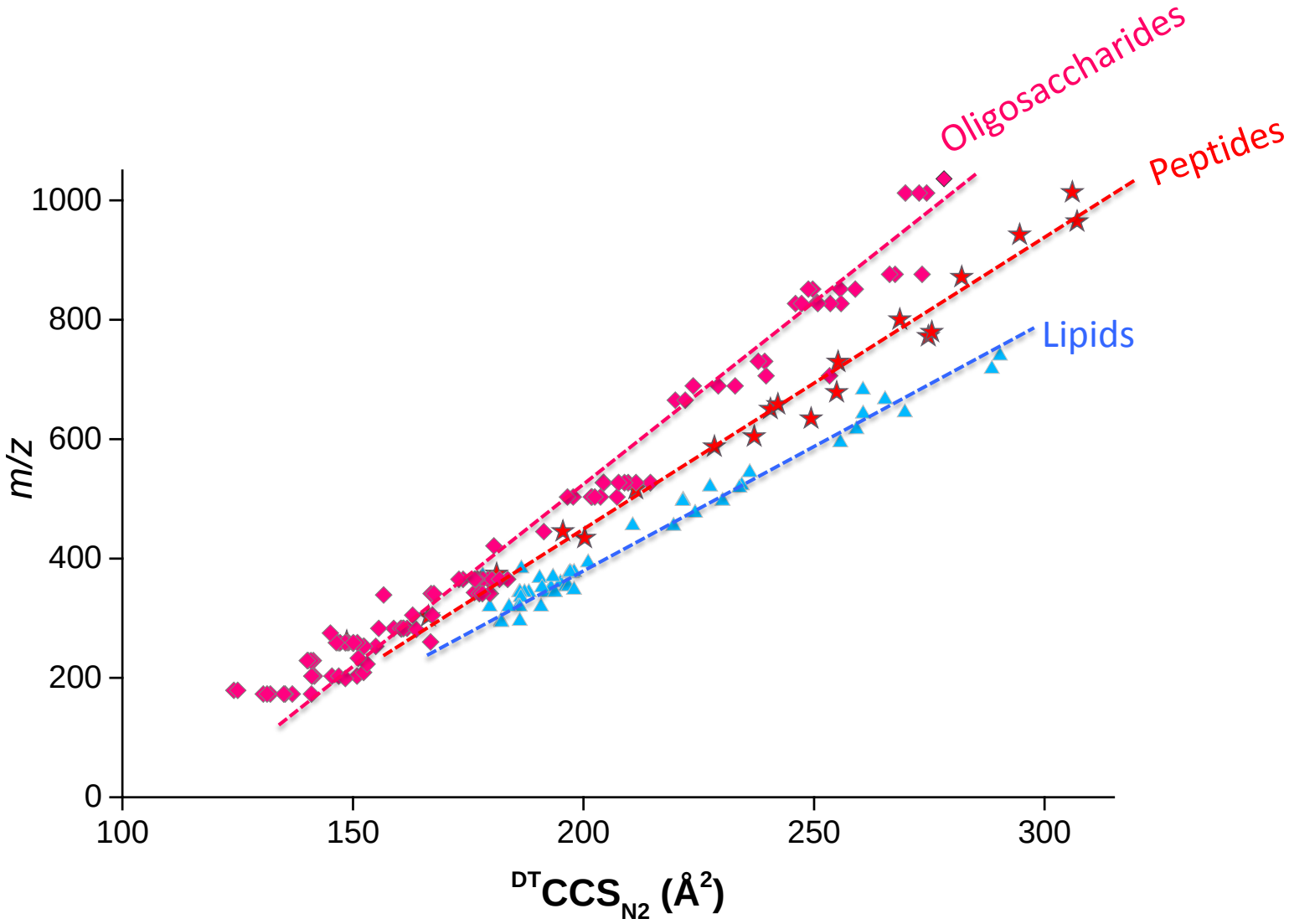
Triplicate measurements
Positive & negative modes

7-voltage CCS Calculations

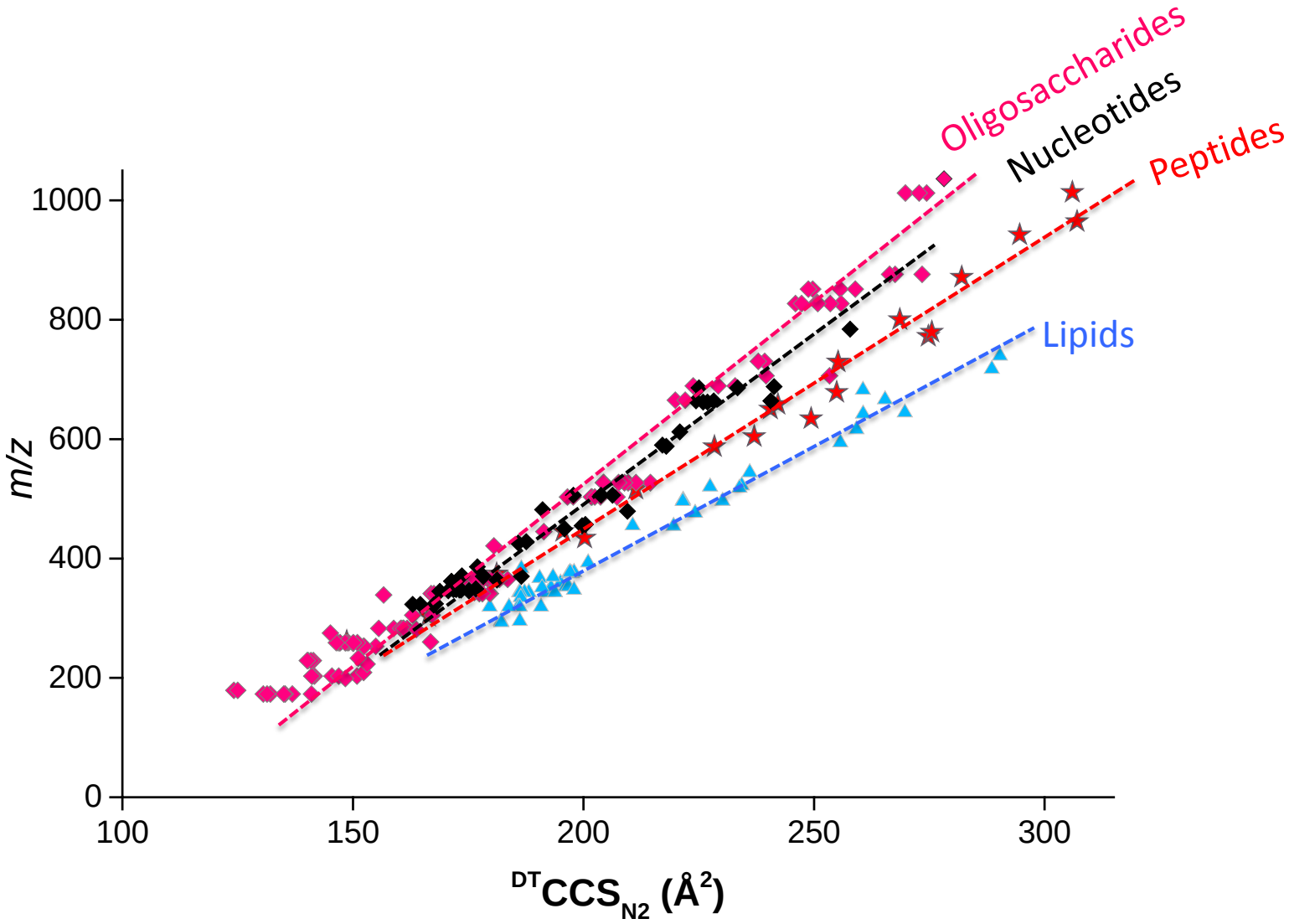


$$\mathrm{CCS} = 1.3\left(\frac{q^2E^2T}{\mu\, k_B p^2 N^2 l^2}\right)^{1/2}(t_A - t_0)$$

Drift tube IMS CCS database



Drift tube IMS CCS database



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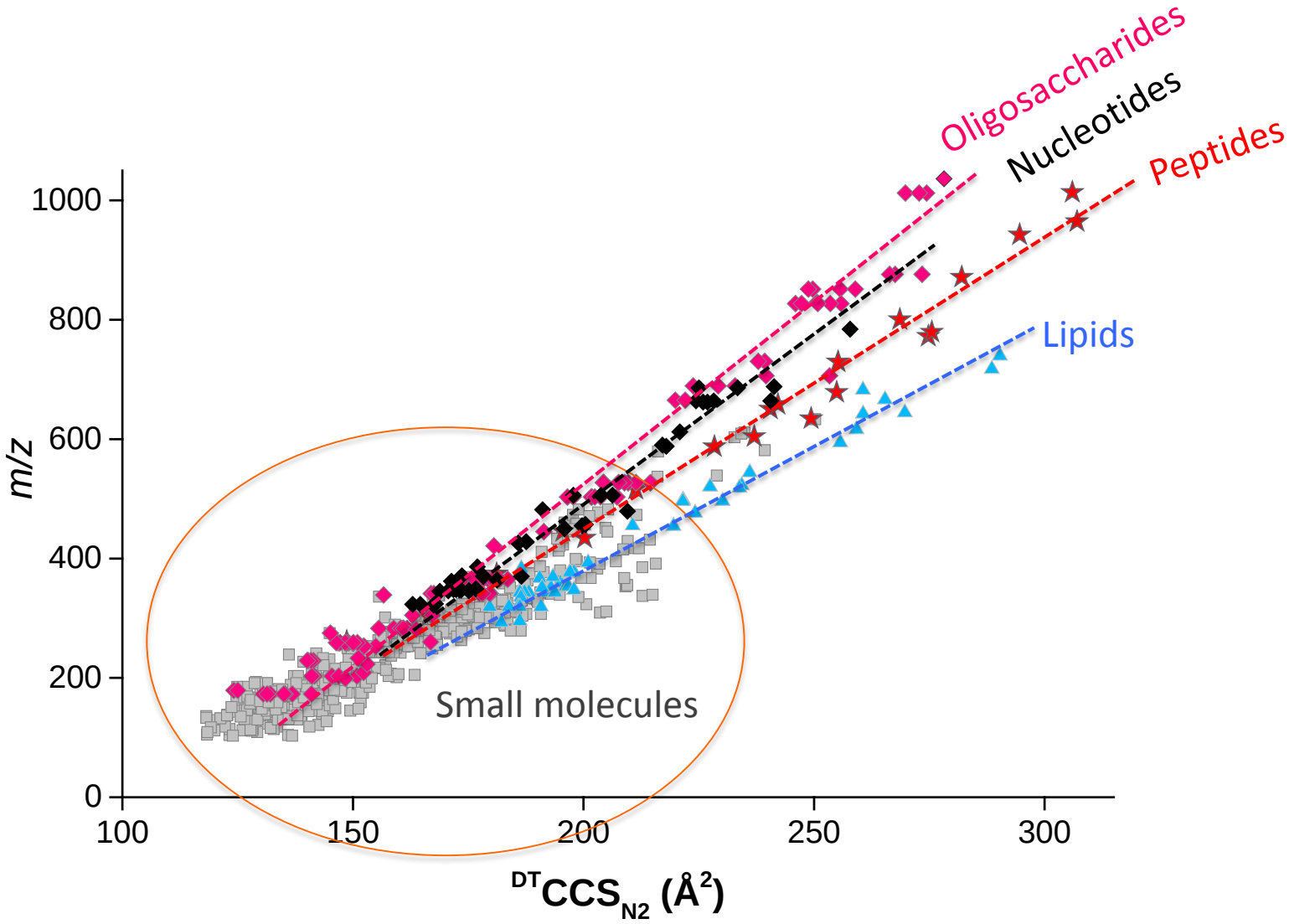

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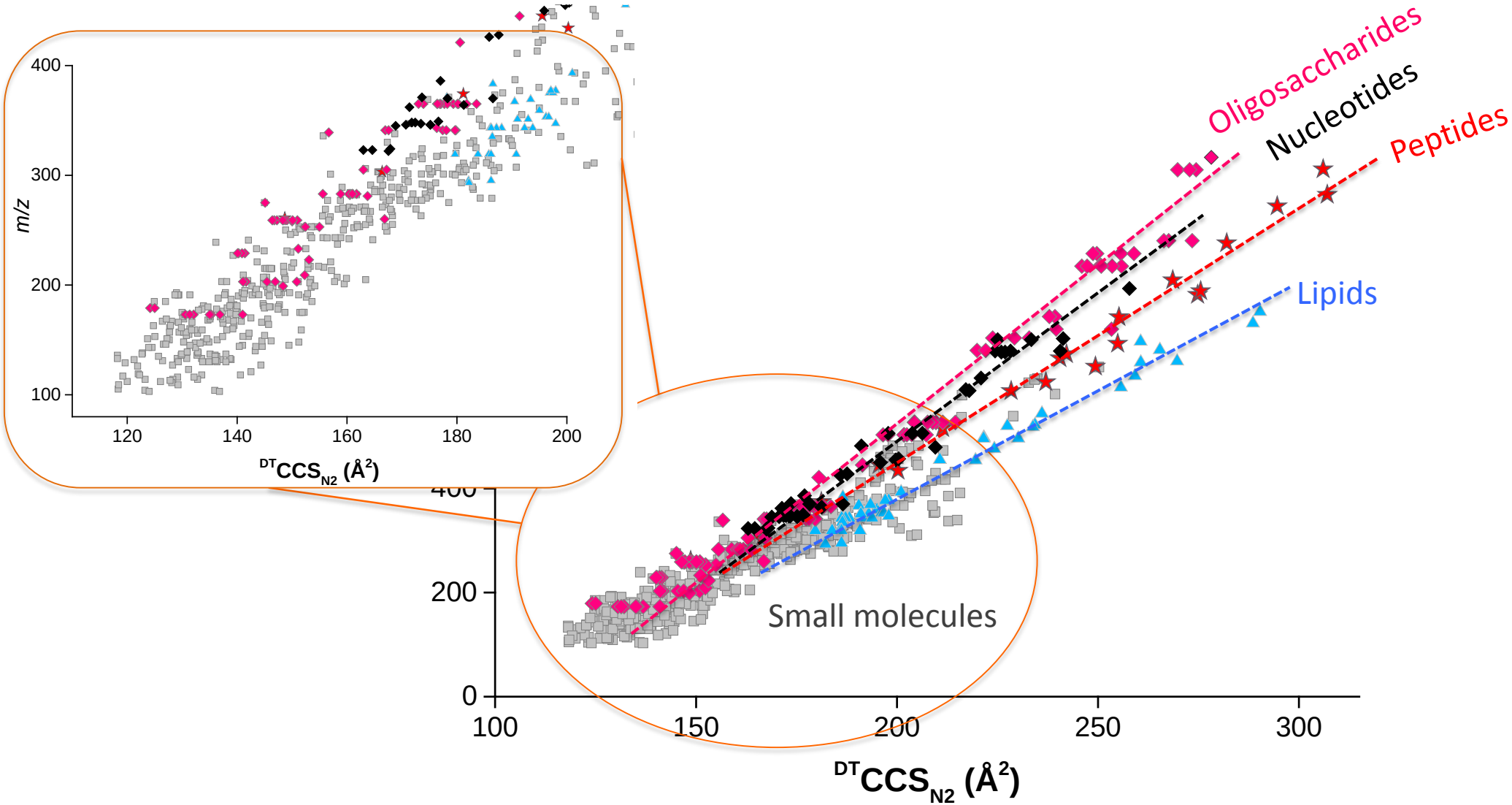
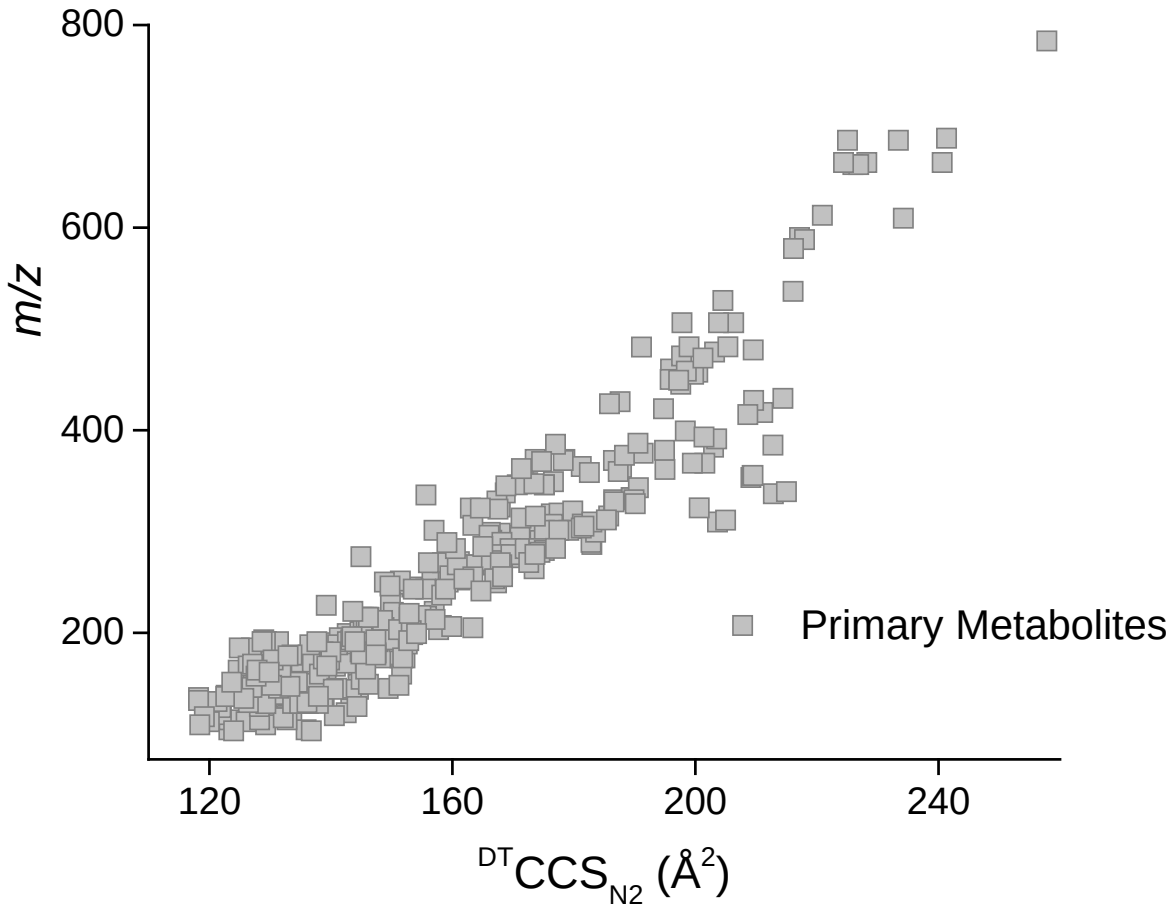
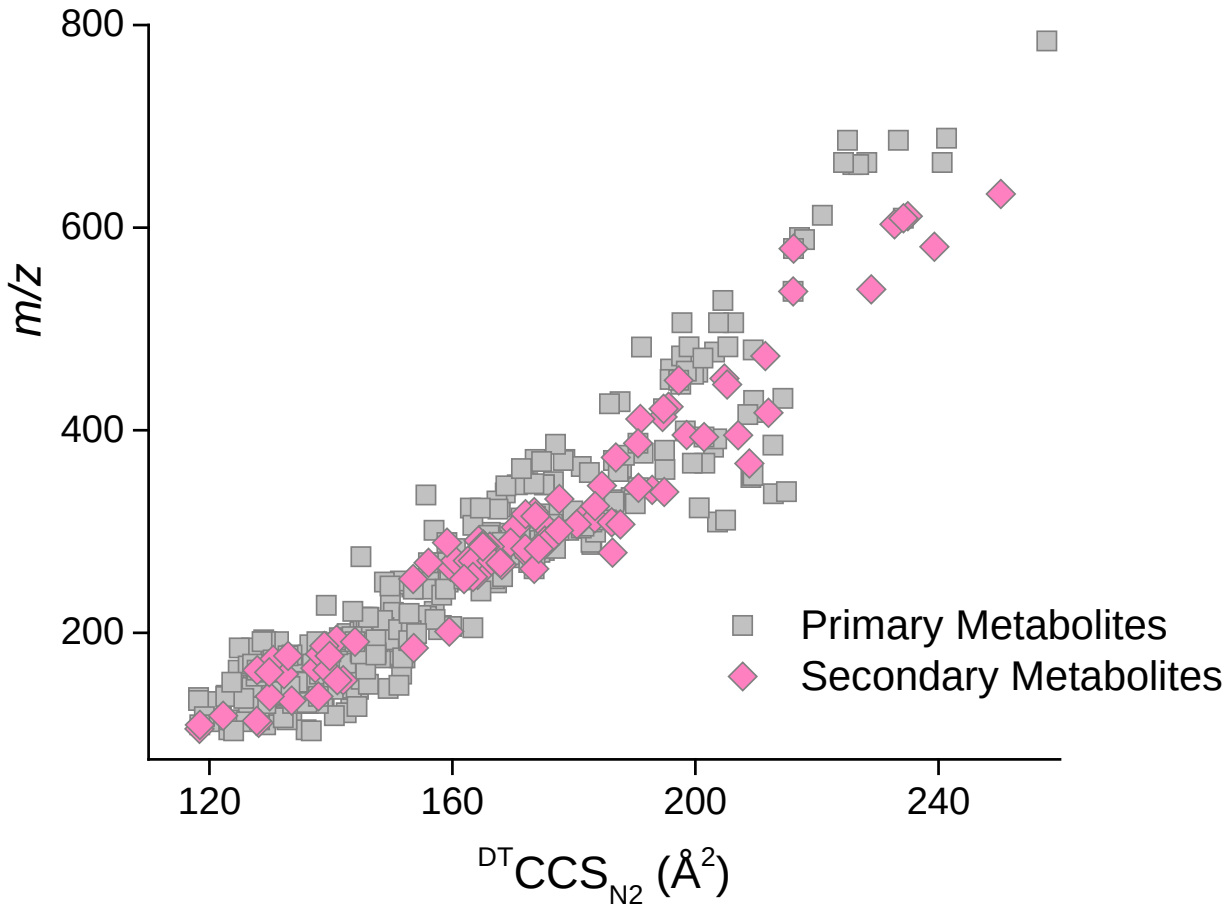


IMS CCS for secondary and xenobiotic metabolites



IMS CCS for secondary and xenobiotic metabolites



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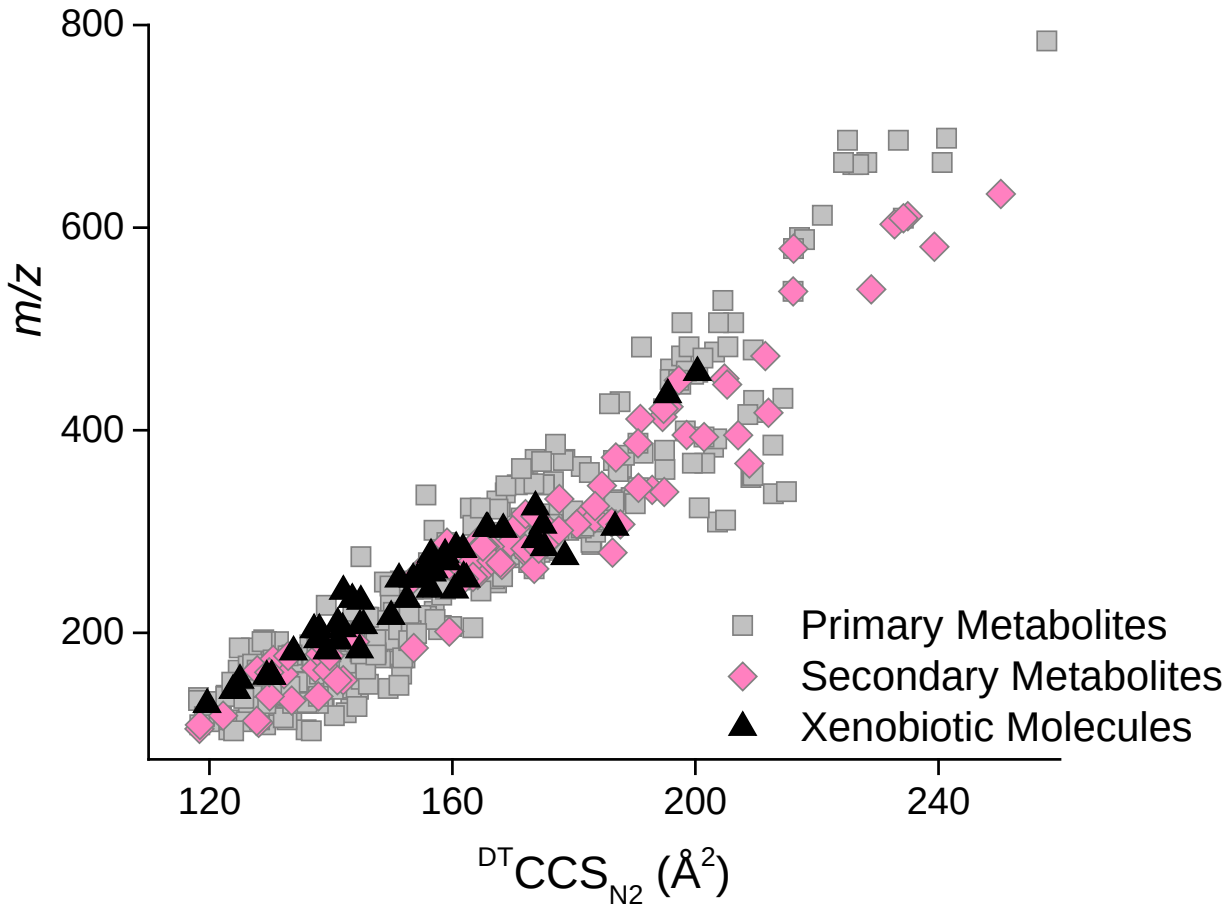
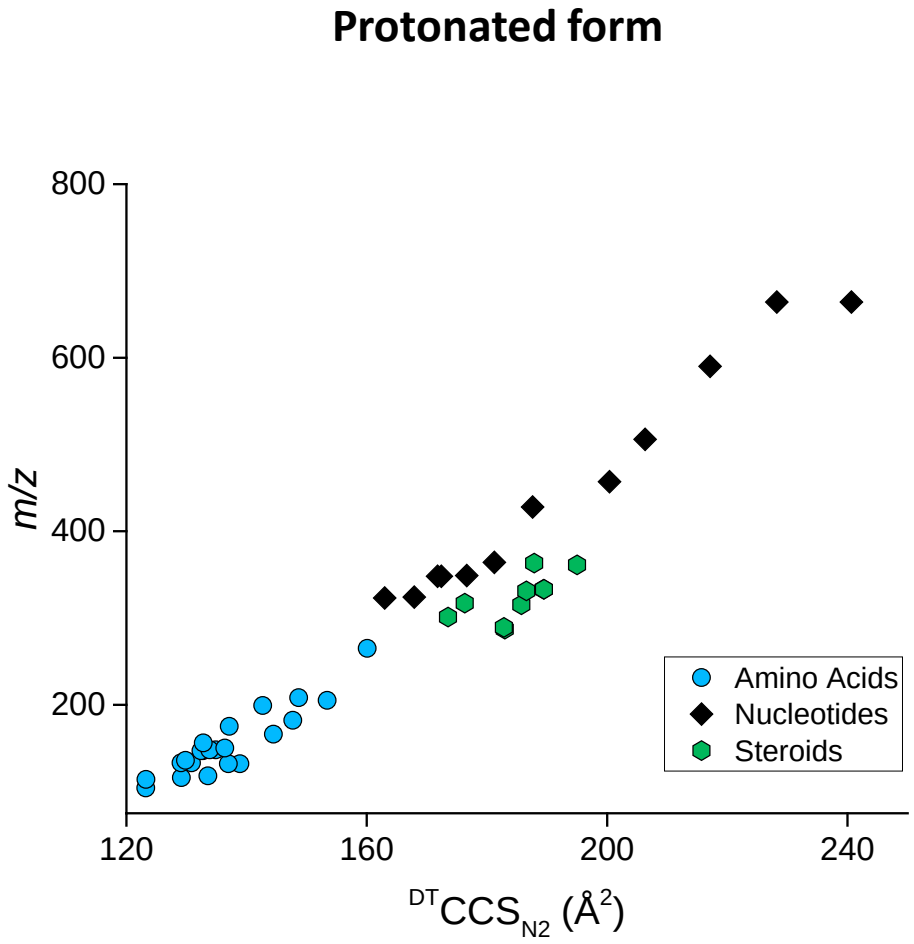


IMS CCS for primary metabolites



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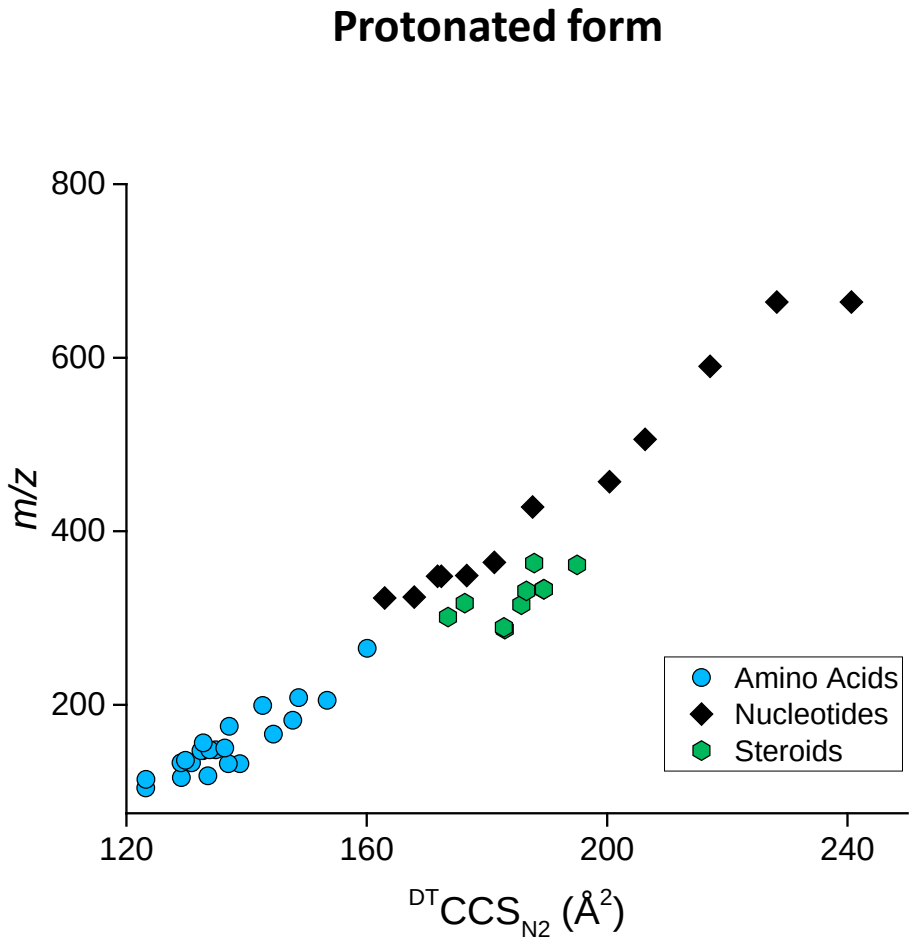
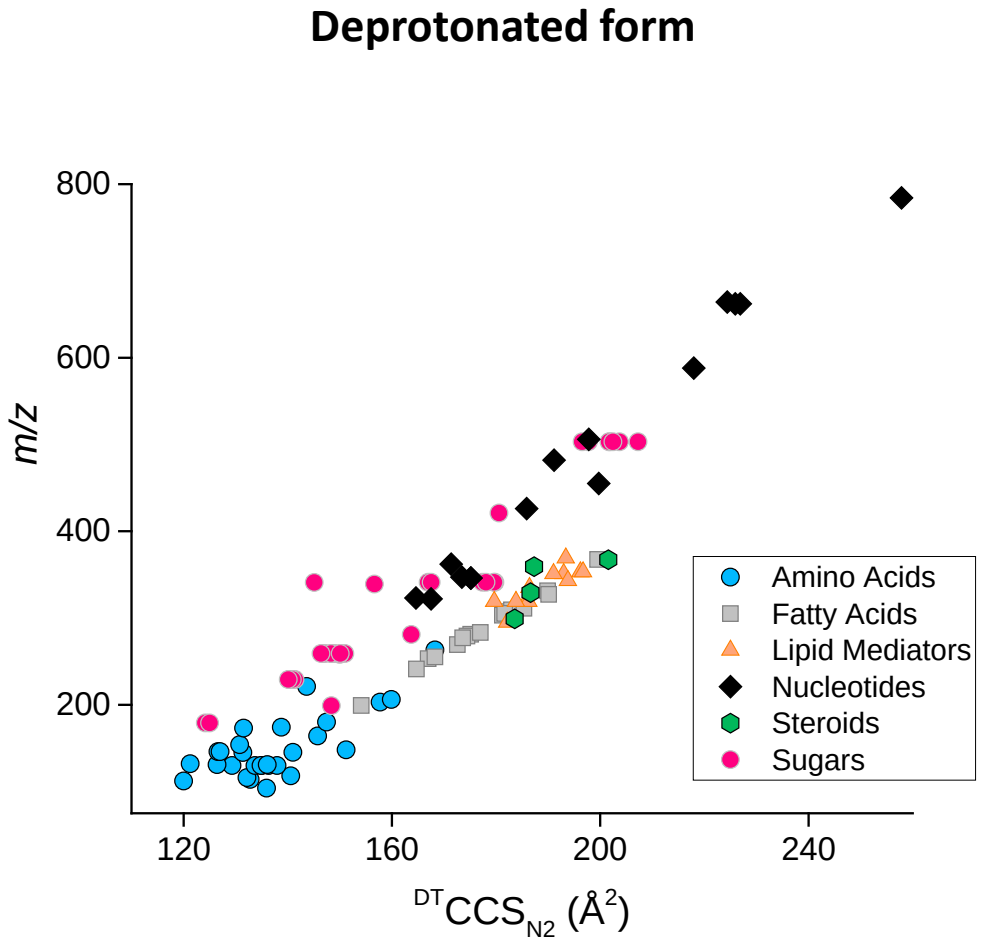
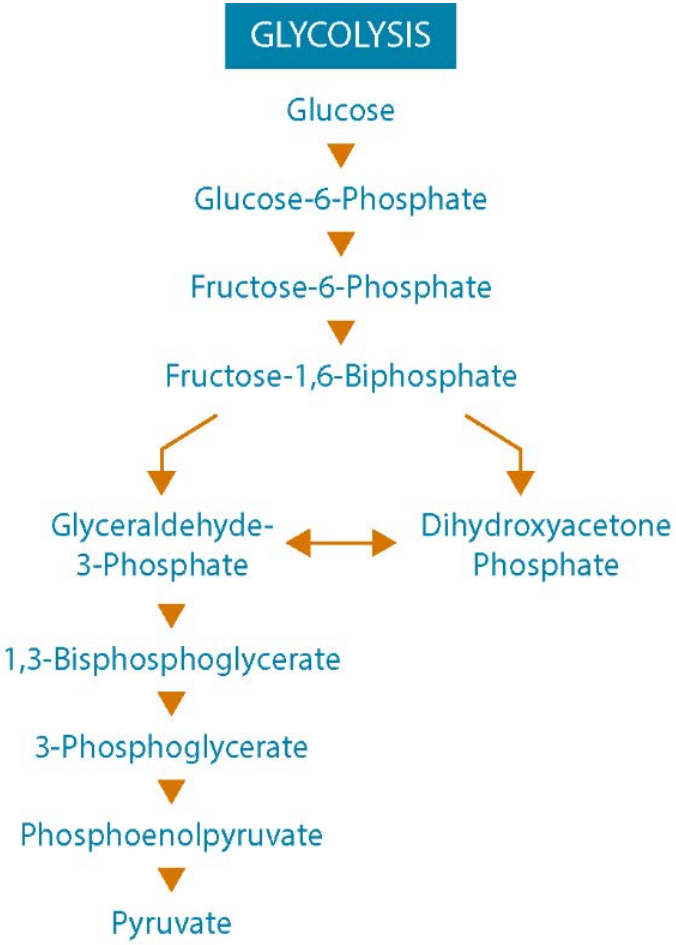
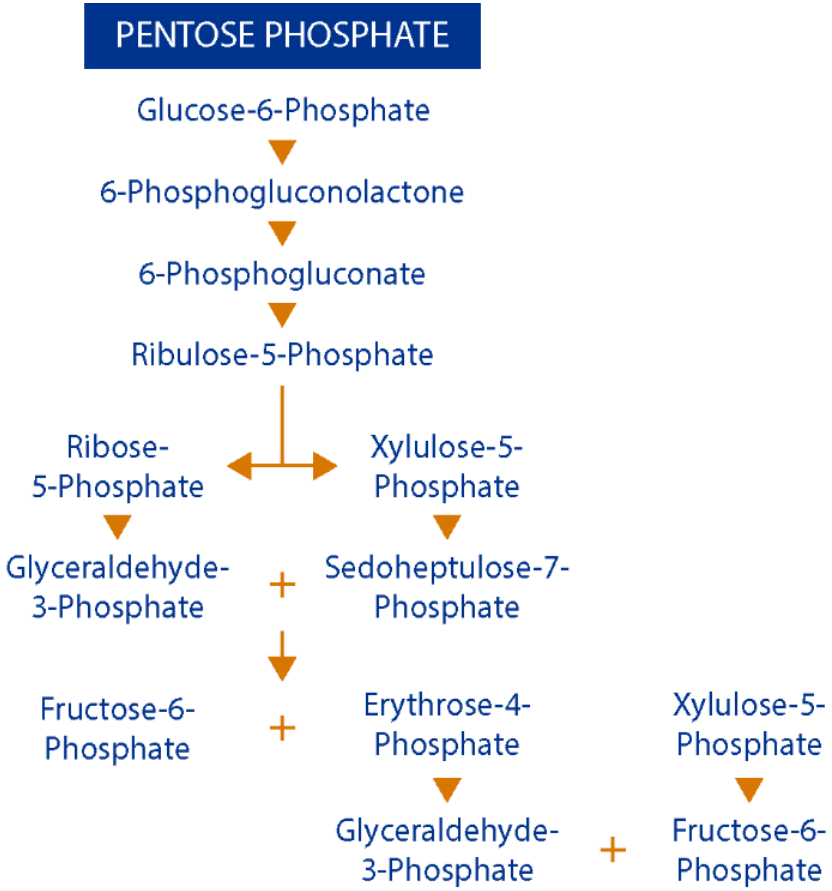
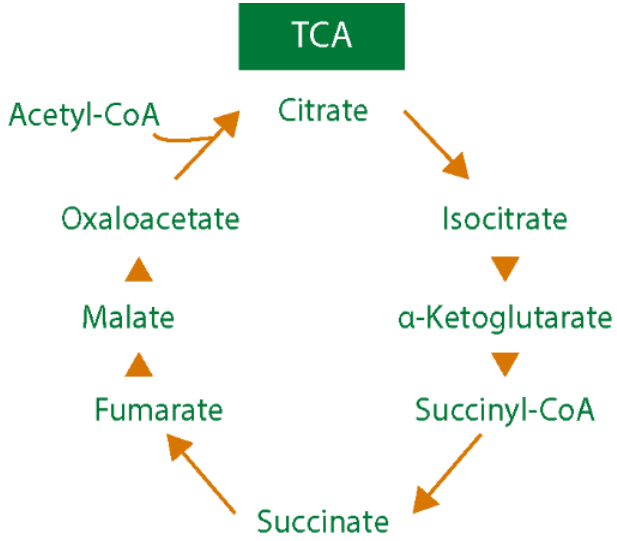


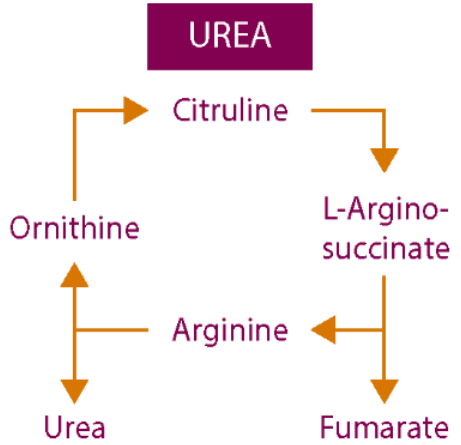


Mapping metabolic pathways





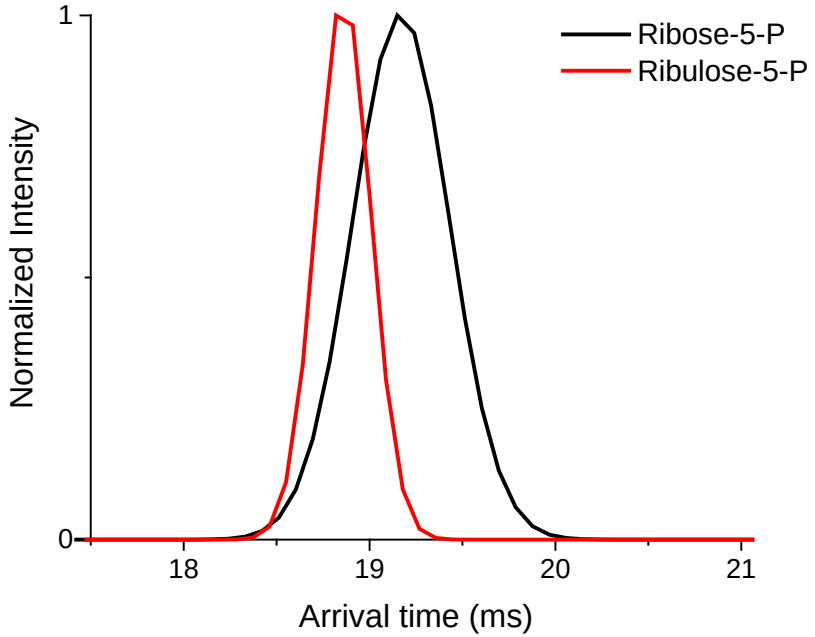




Isomeric separation

Ribulose 5-P

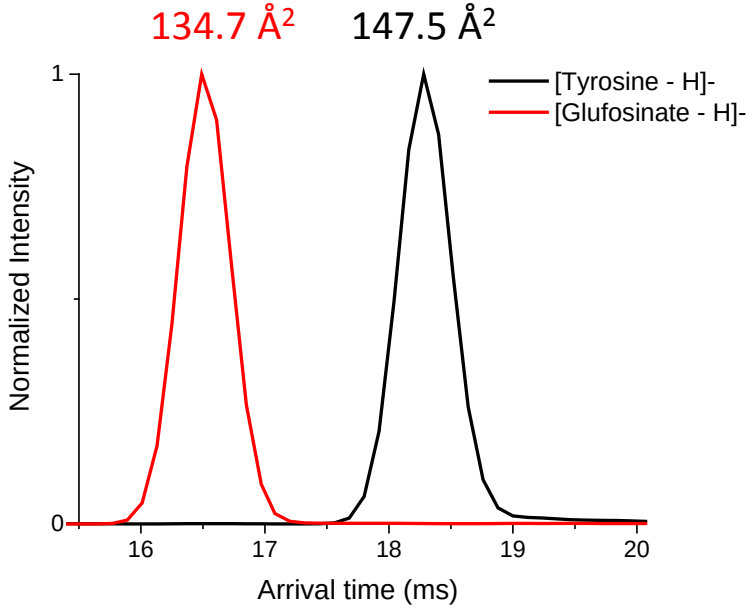
Ribose 5-P



Distinguishing molecules with the same nominal mass

L-Tyrosine (amino acid)
Exact mass: 181.0738896

Glufosinate (herbicide)
Exact mass: 181.0503922

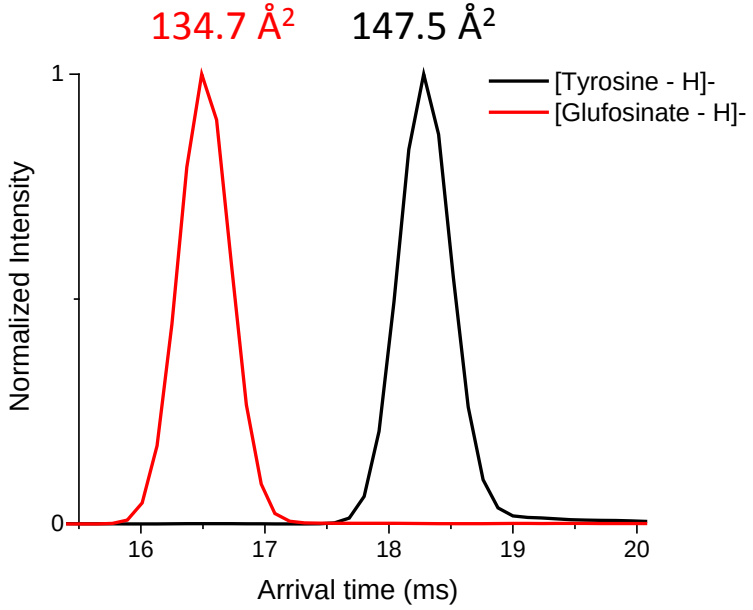


Deprotonated form

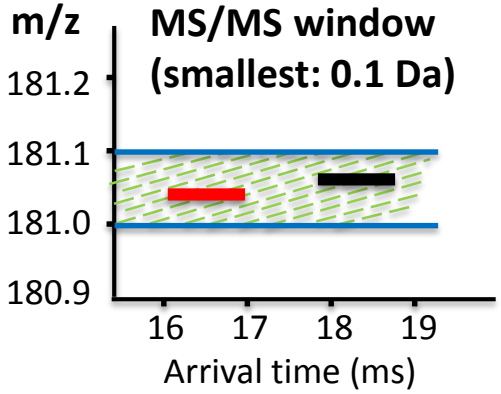
Distinguishing molecules with the same nominal mass

L-Tyrosine (amino acid)
Exact mass: 181.0738896

Glufosinate (herbicide)
Exact mass: 181.0503922



Deprotonated form

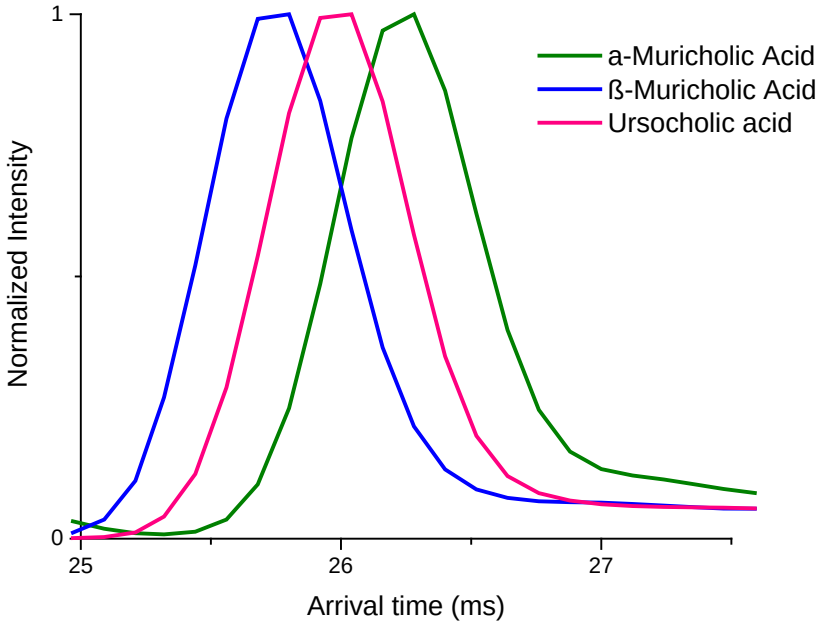


Identification of unknown bile acid metabolite from microbiome

α-Muricholic Acid

β-Muricholic Acid

Ursocholic acid

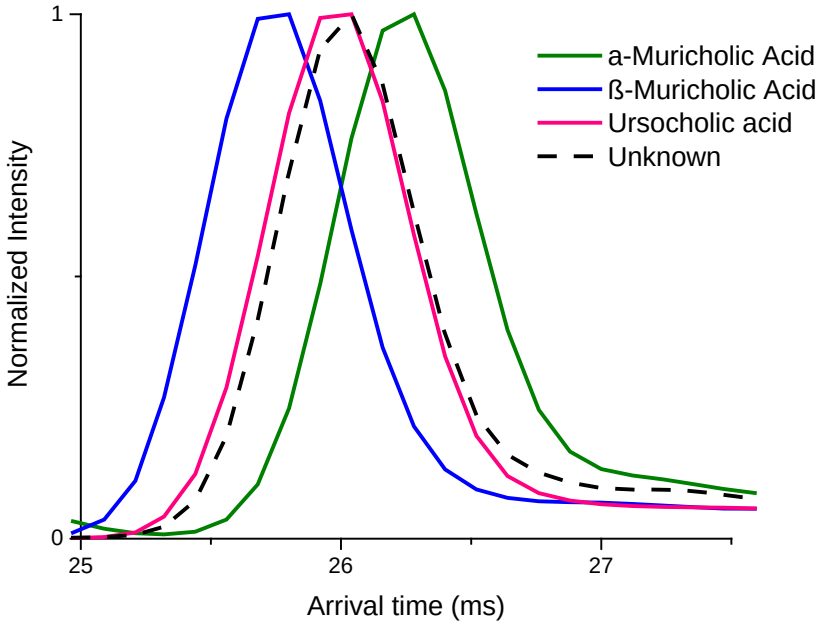


$[M - H]^-$, m/z = 407.28

Identification of unknown bile acid metabolite from microbiome

α-Muricholic Acid

β-Muricholic Acid



[M - H]$^-$, m/z = 407.28

Ursocholic acid

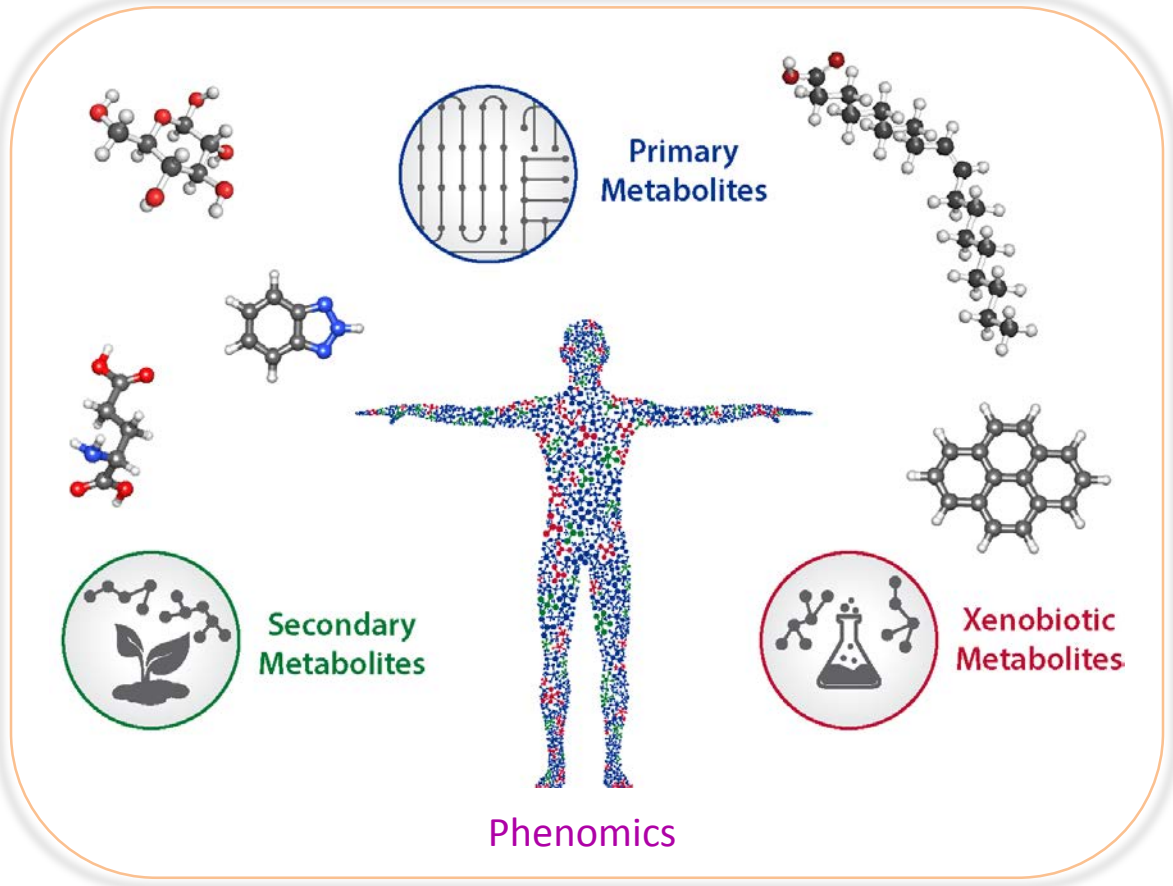
Further validated by NMR

Compound	CCS (Å^2)
α-Muricholic Acid	206.33 ± 0.25
β-Muricholic Acid	201.93 ± 0.48
Ursocholic Acid	204.00 ± 0.62
Unknown	203.97 ± 0.20

Summary

- Using IMS-MS and CCS database, we hope to better understand present and changing small molecules.
- We have created a website with all CCS database and structural information for public release as soon as our paper is accepted.

CCS database for small molecules



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Omics

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