Pacific Northwest
NATIONAL LABORATORY
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Rapid sample analyses for environmental toxicants

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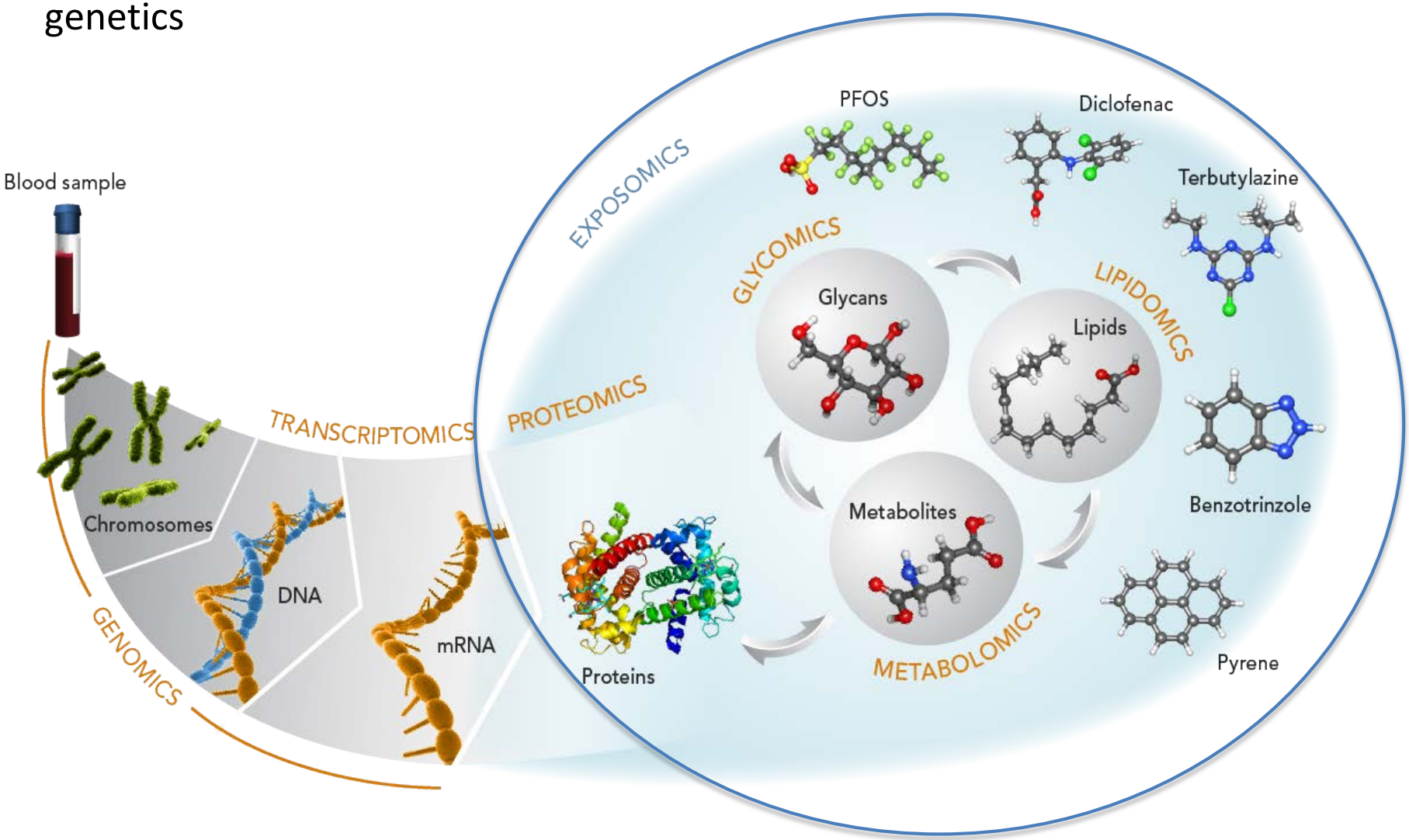
Xueyun Zheng, Noor A. Aly, Francesca B. Smith, Kristin E. Burnum-Johnson, Samuel H. Payne, Matthew E. Monroe, Richard D. Smith

Pacific Northwest National Laboratory

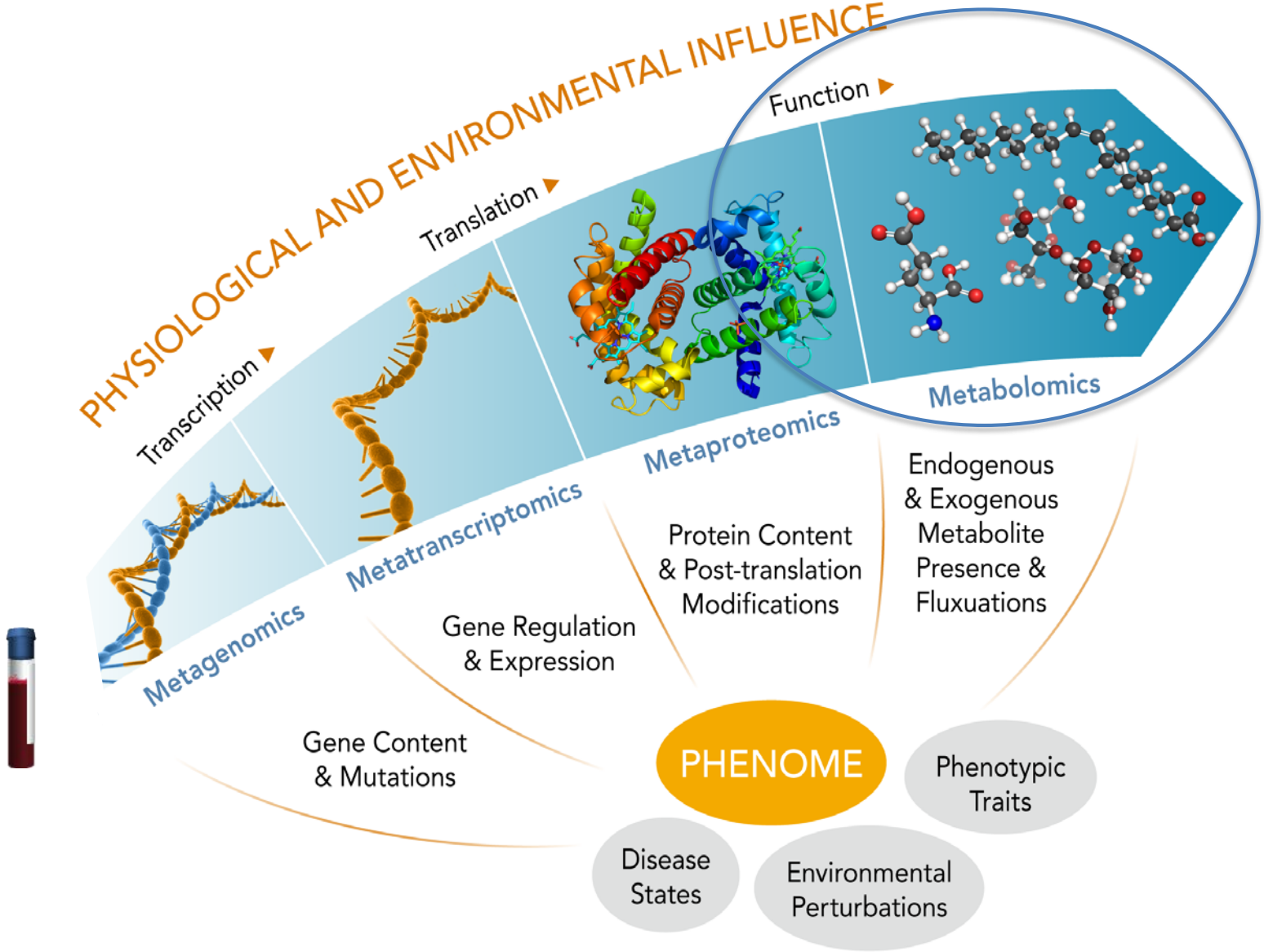
Multi-omic Future

- Human genome project illustrated that >90% of diseases are not due solely to genetics



MS-based Measurements

Small molecule enable a functional understanding



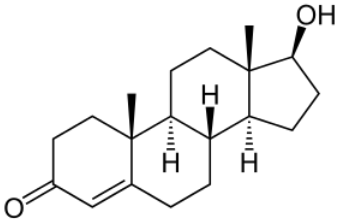
Main challenges with small molecule measurements

1. Small molecules of interest occur from very low to high concentrations (fM-mM) so measurements with high dynamic range and sensitivity are essential

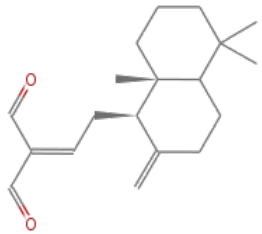
2. Biological changes are best understood when both endogenous metabolites and xenobiotics are analyzed

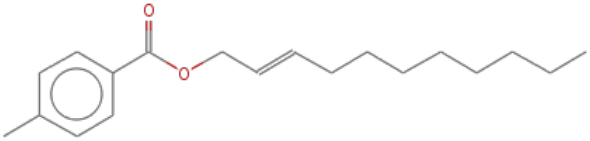
3. Untargeted measurements covering thousands of small molecules are desired to perform time course studies and analyze large cohorts

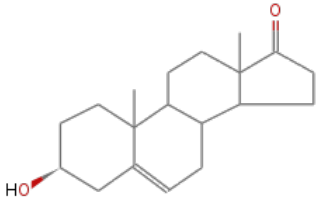
4. Many small molecules have the same masses but a different chemical makeup so distinguishing them with MS-based approaches can be difficult or impossible



Testosterone
Exact mass = 288.2089188

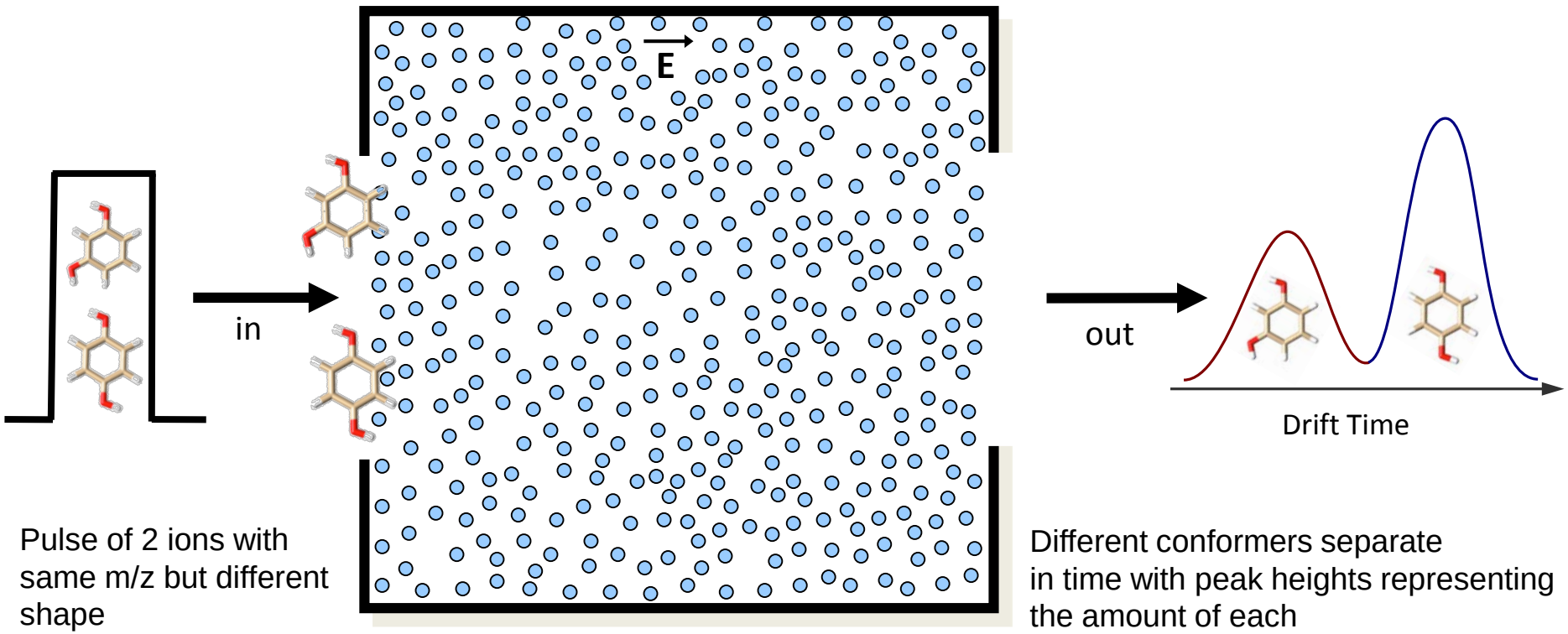






In the NIST database there are
18 different options with exact mass = 288.2089188

Ion mobility concept



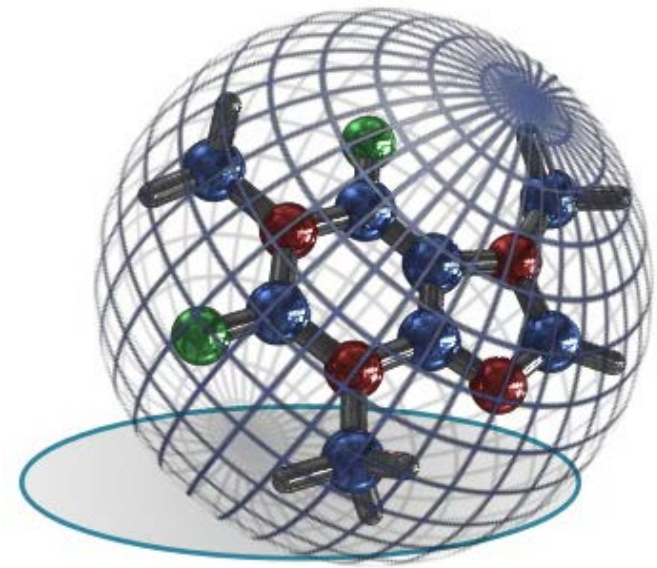
velocity is constant

$$v = K \vec{E}$$

K = ion mobility

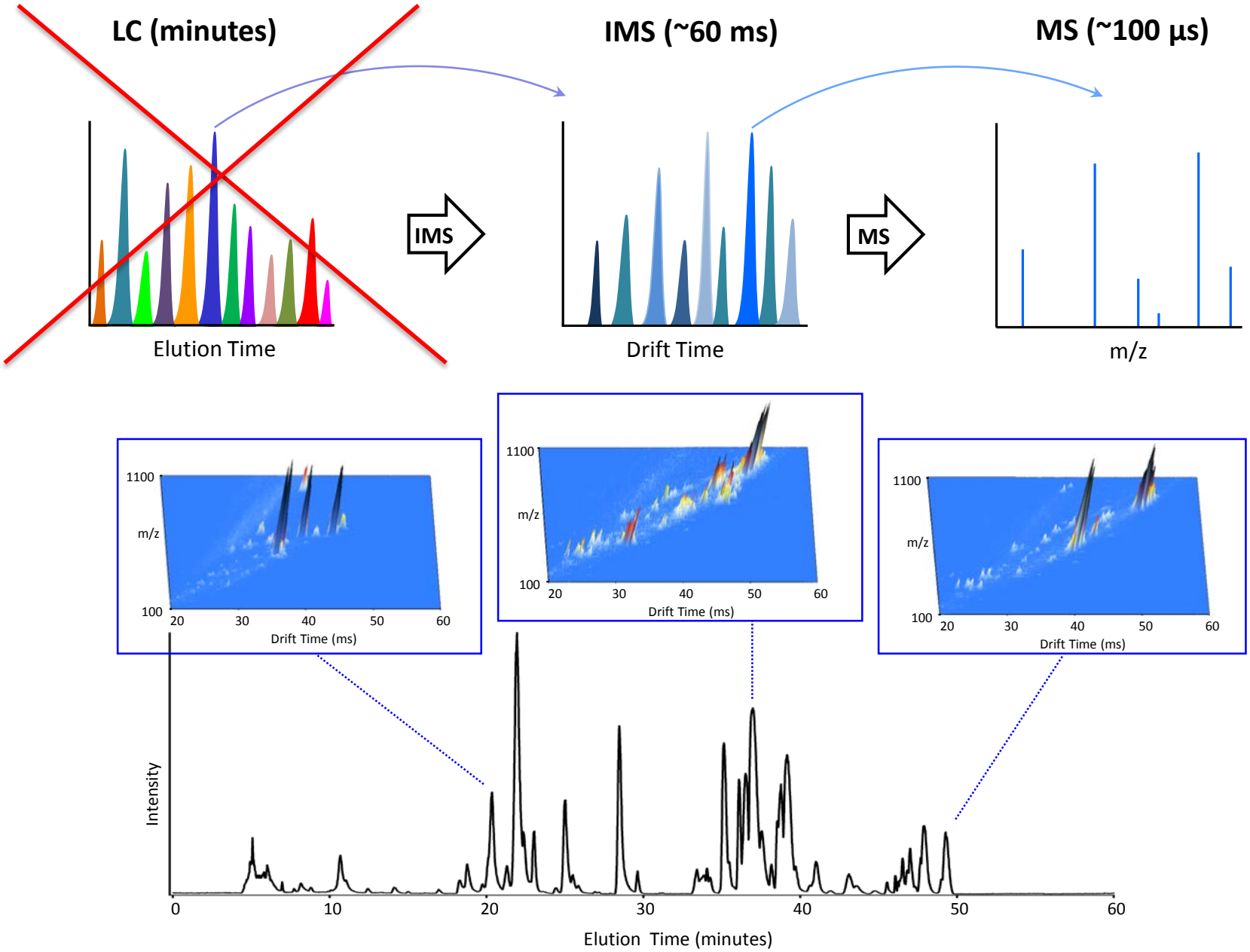
Ion-neutral collision cross section (CCS)

1. Value related to the size and shape of an ion
2. Corresponds to the area that collides with the drift gas
3. Robust physicochemical property
4. Can easily be compared between labs
5. Varies depending on drift gas

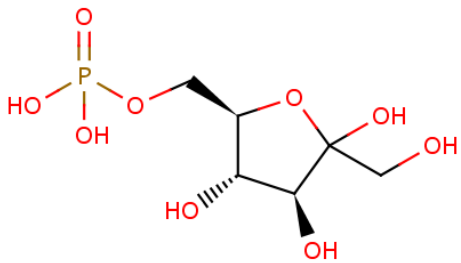


Slide courtesy of Professor Kevin Pagel and Waters

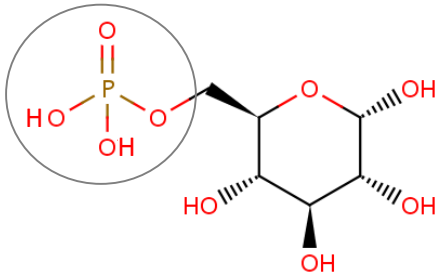
Ion mobility concept



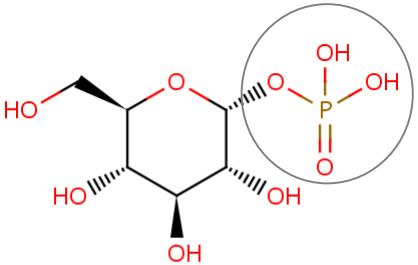
Isomers difficult to separate with hydrophobic interaction liquid chromatography (HILIC)



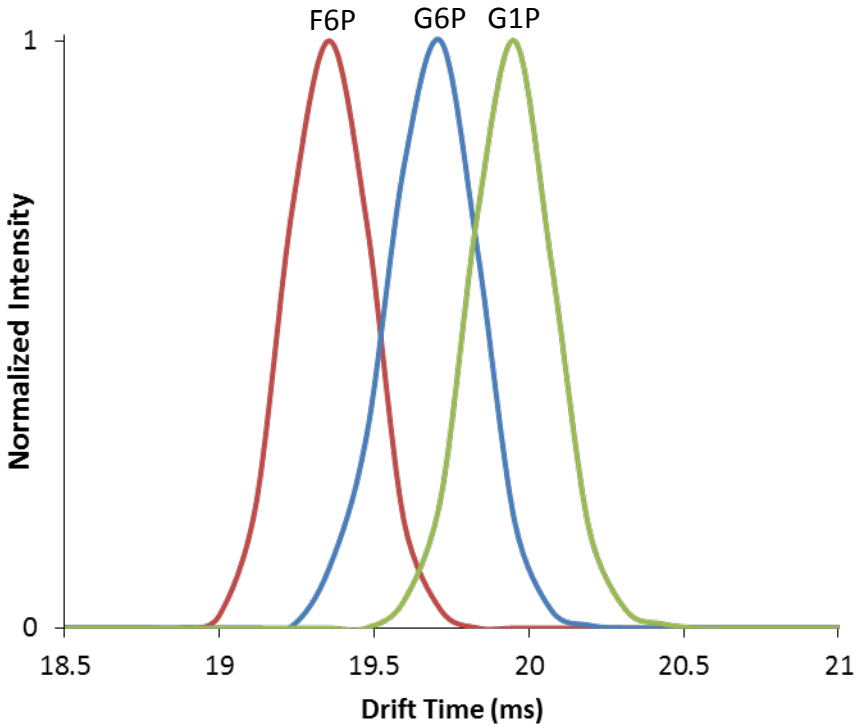
D-Fructose-6-phosphate (F6P)

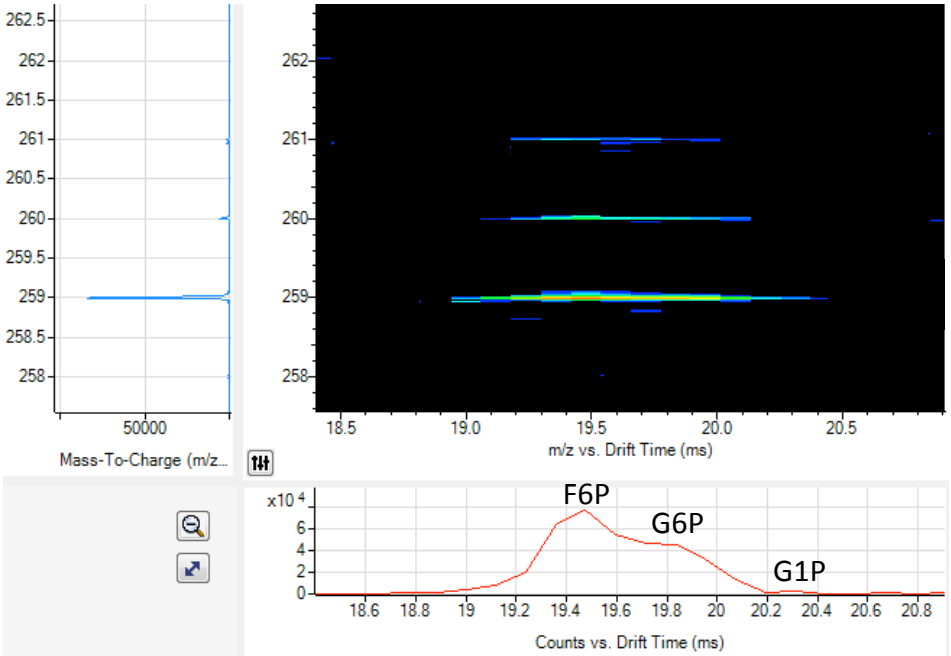


D-Glucose-6-phosphate (G6P)



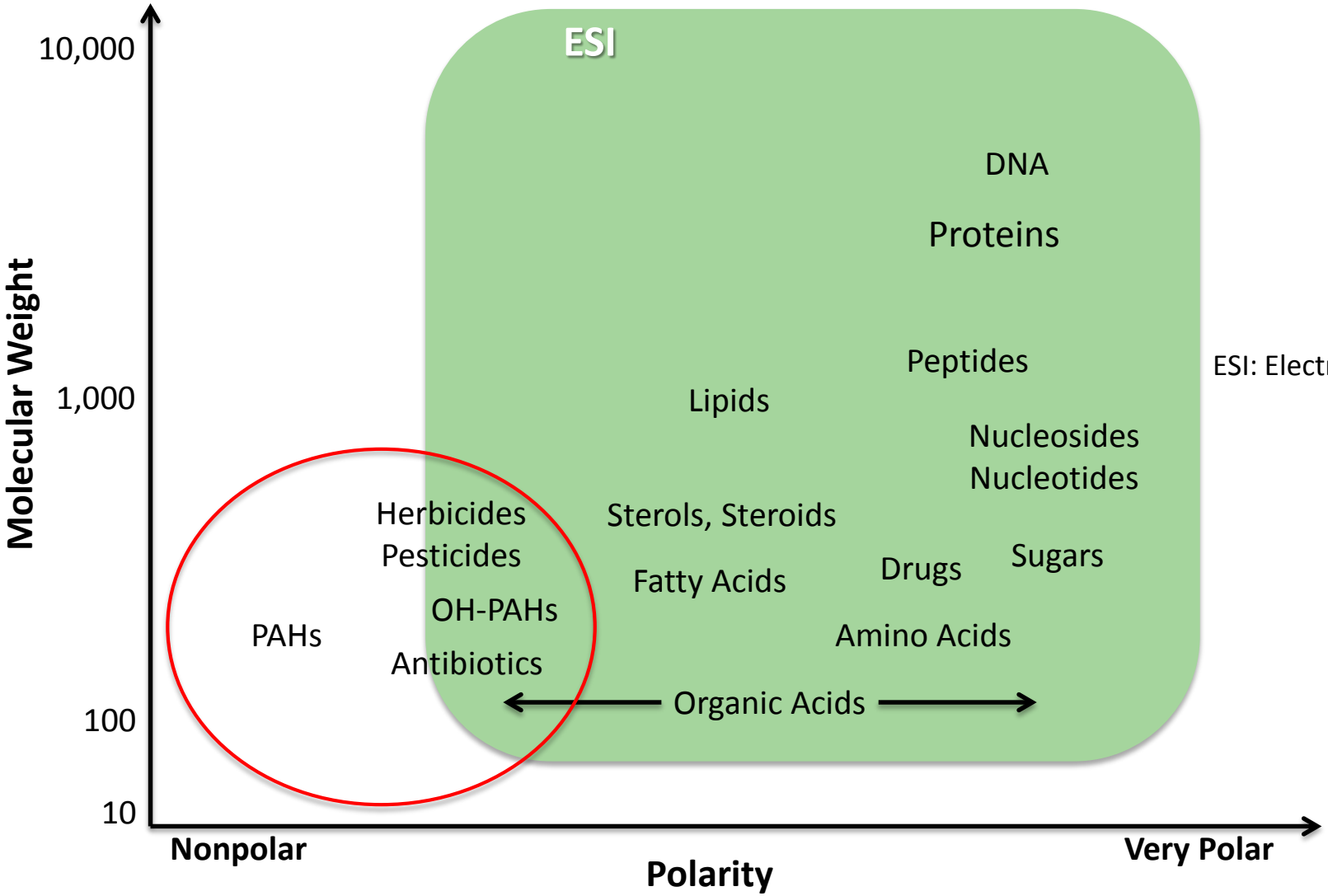
α-D-Glucose-1-phosphate (G1P)





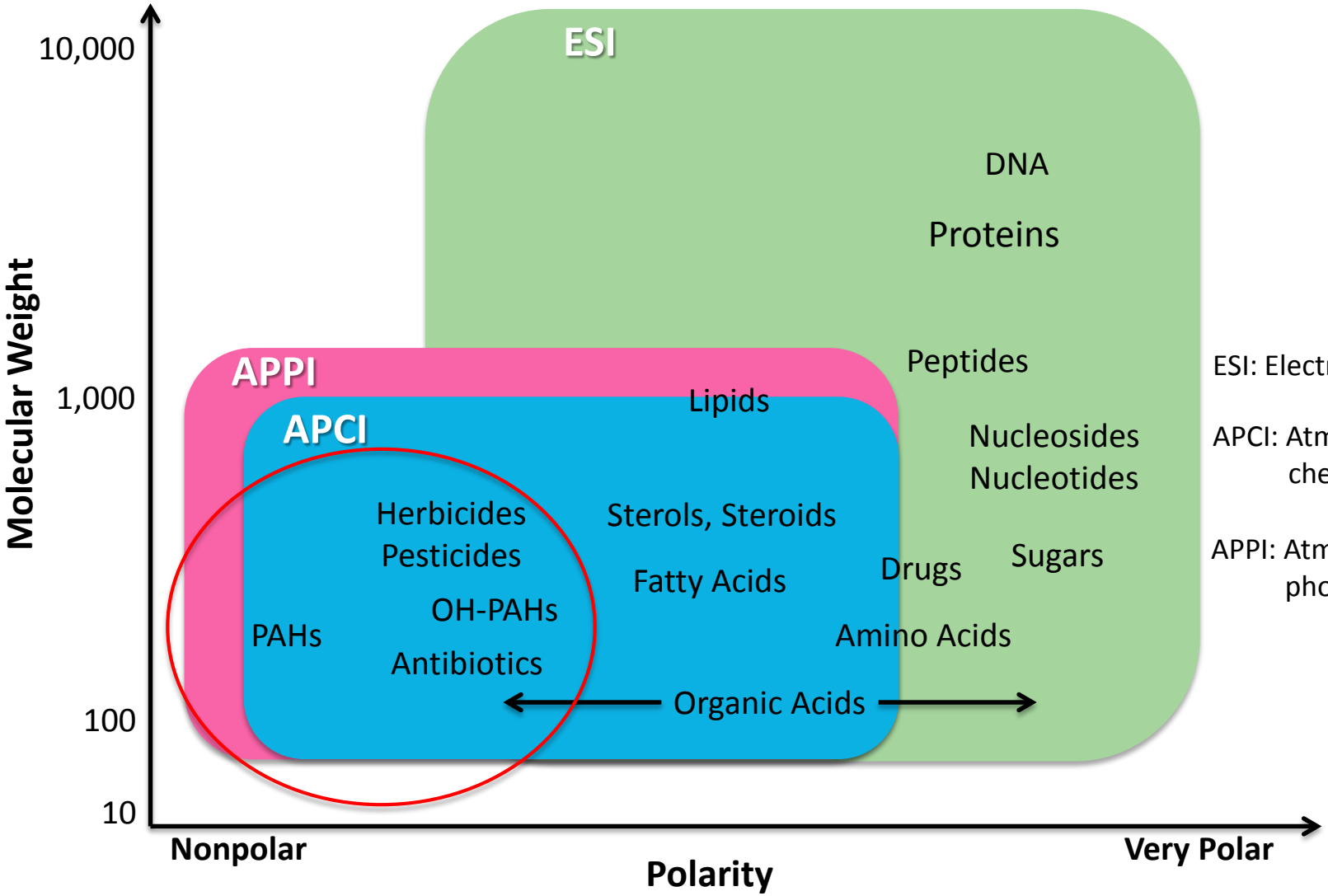
Deprotonated form $[M - H]^-$ m/z = 259.02

Different ionization methods



ESI: Electrospray ionization

Different ionization methods



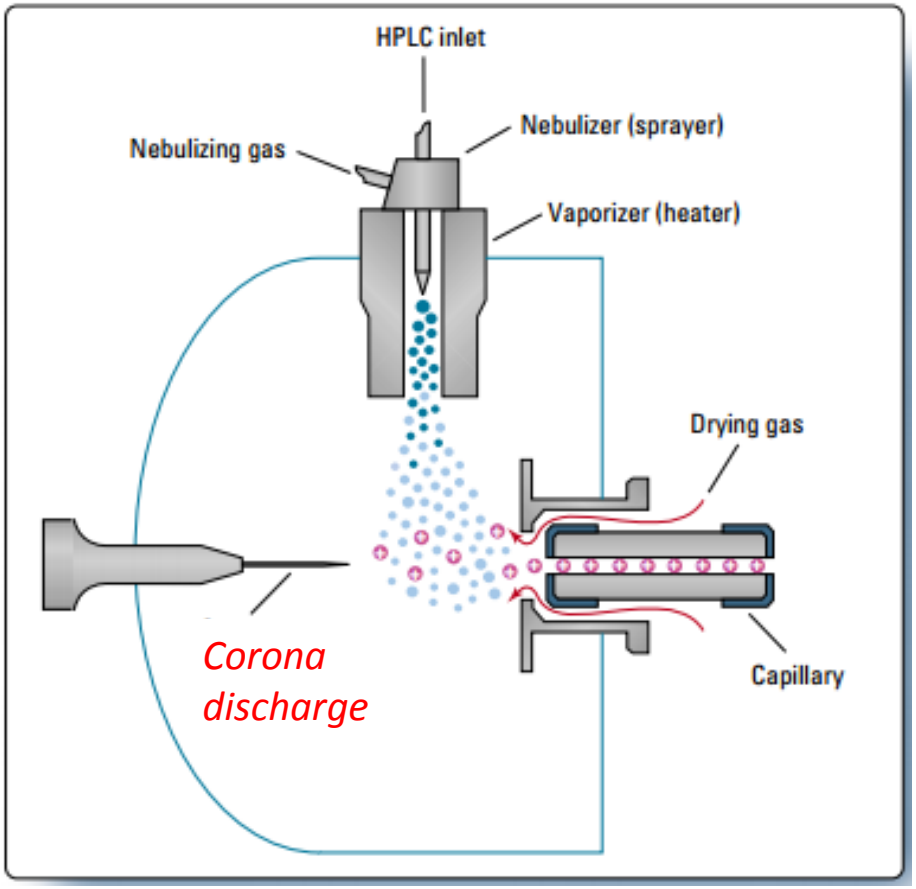
ESI: Electrospray ionization

APCI: Atmospheric pressure chemical ionization

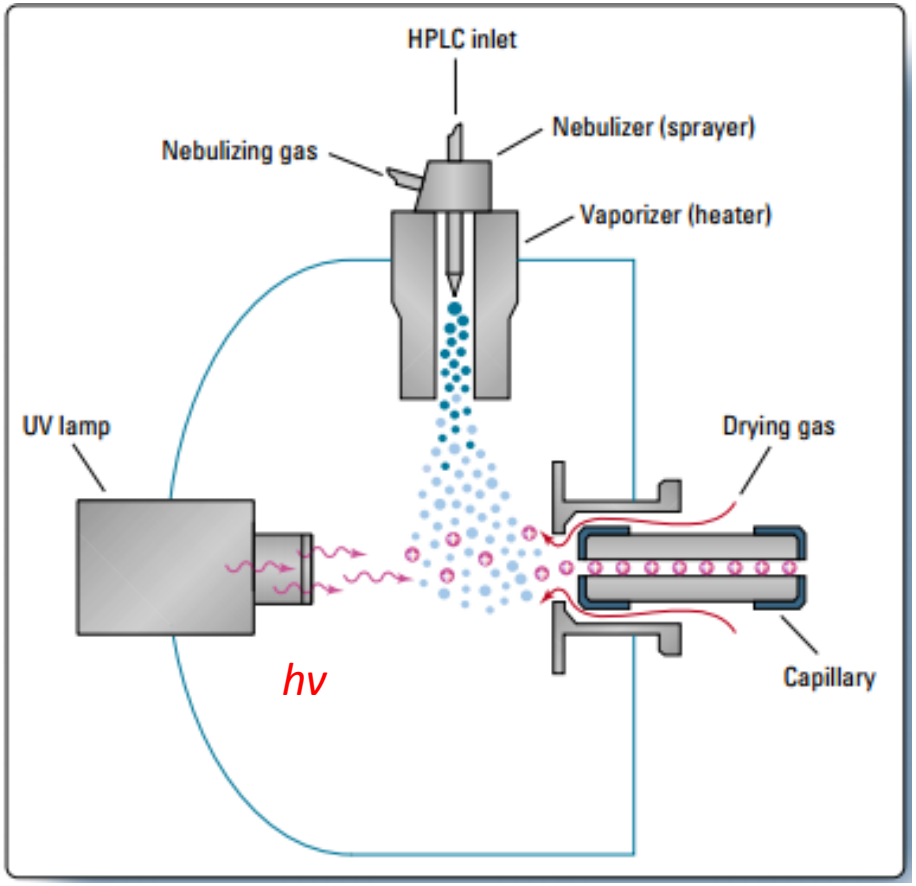
APPI: Atmospheric pressure photoionization

Different ionization methods

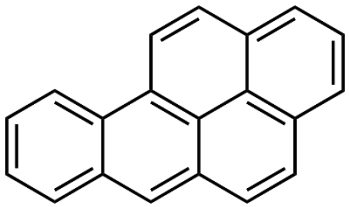
APCI
Atmospheric pressure chemical ionization



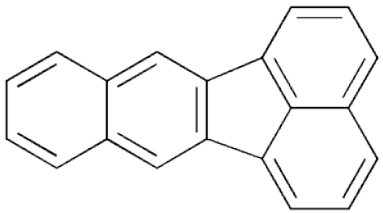
APPI
Atmospheric pressure photoionization



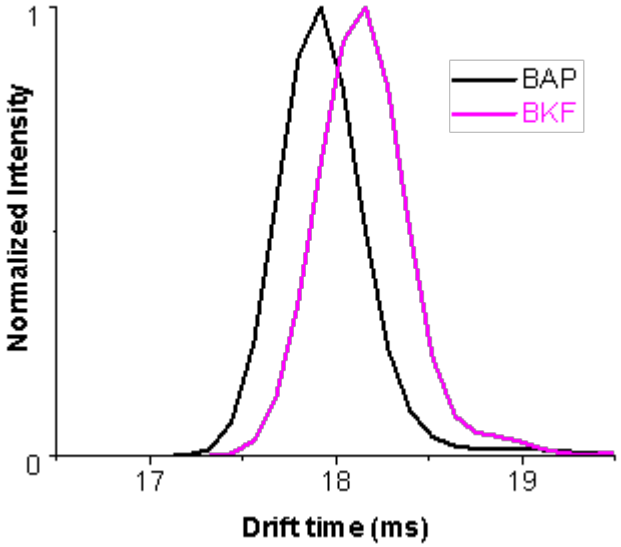
Isomeric xenobiotic separations



BAP:
Benzo(a)Pyrene

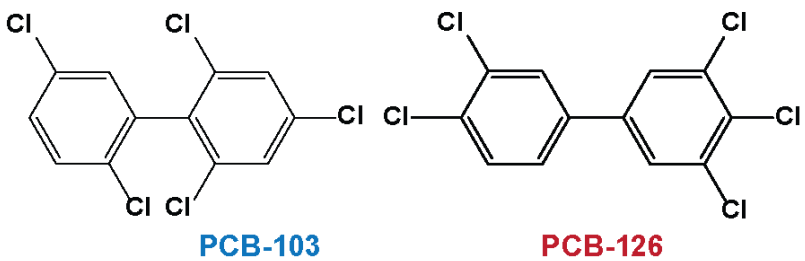


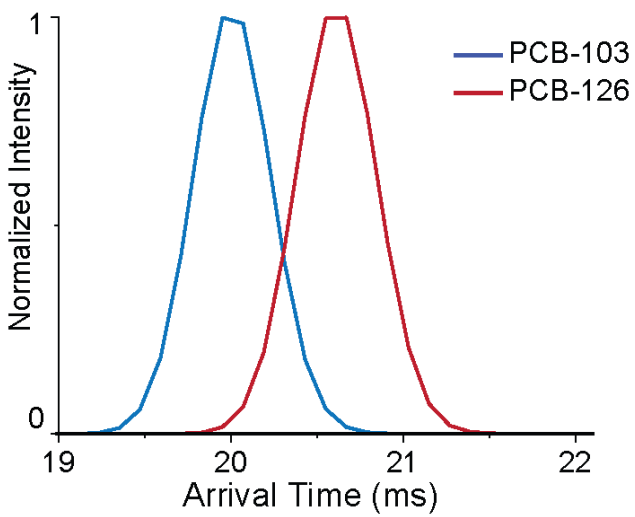
BKF:
Benzo(k)fluoranthene



Radical form $[M]^{\cdot+}$ m/z = 252.09

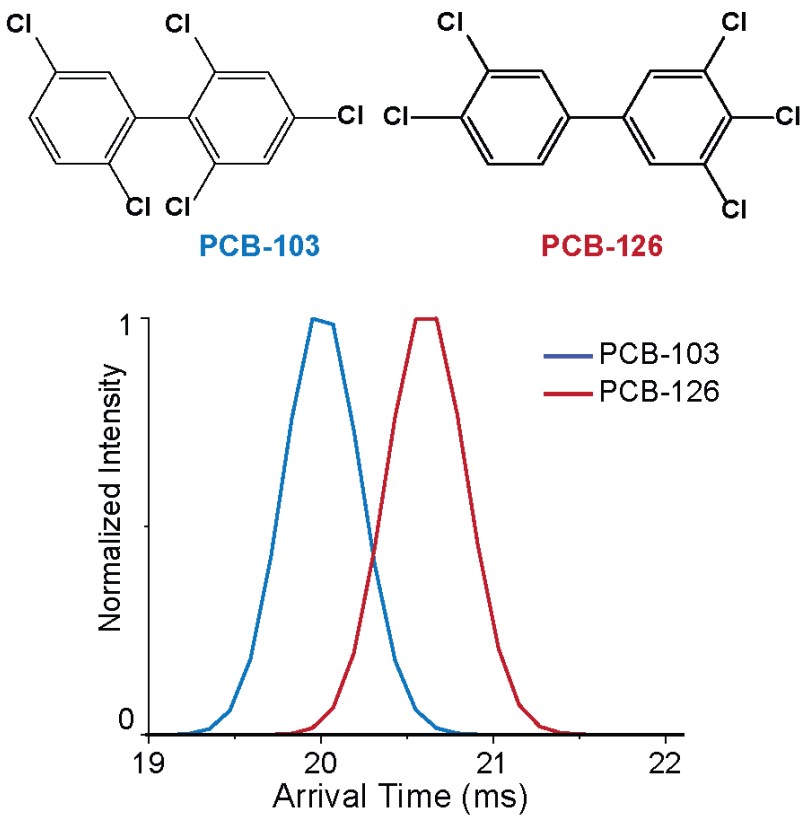
Isomeric xenobiotic separations

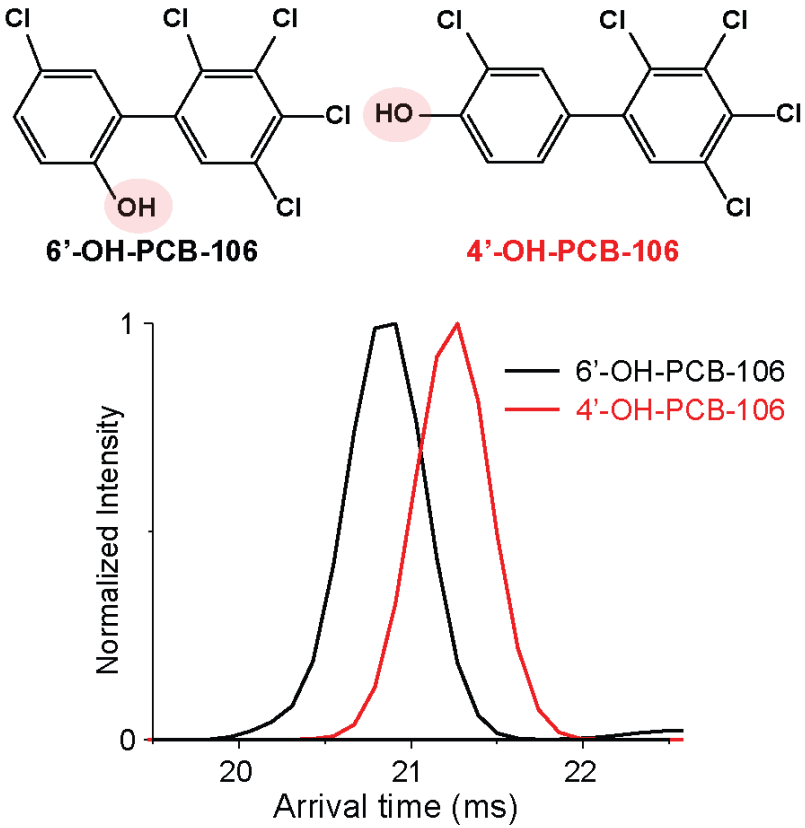




Collaboration with Keri Hornbuckle's Group

Isomeric xenobiotic separations

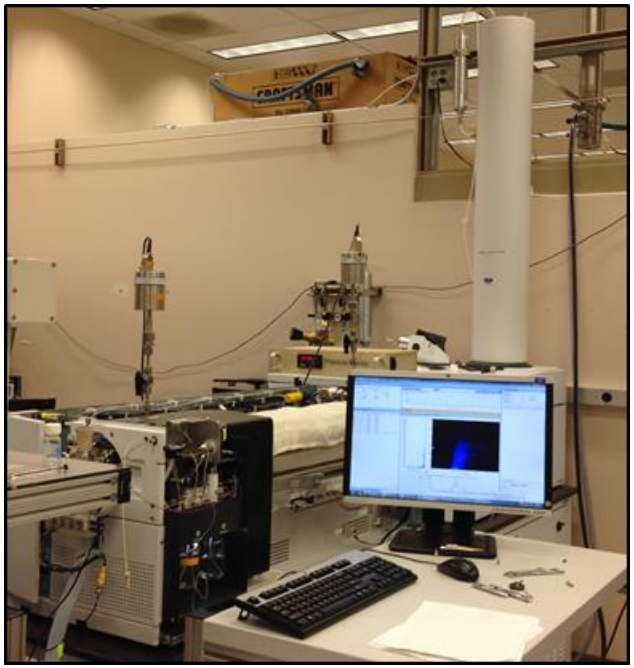




Collaboration with Keri Hornbuckle's Group

IMS collision cross section (CCS) precision

- Compare CCS accuracy across 4 international labs
- Analyze 80 molecules (metabolites, lipids, peptides and proteins) to determine CCS agreement

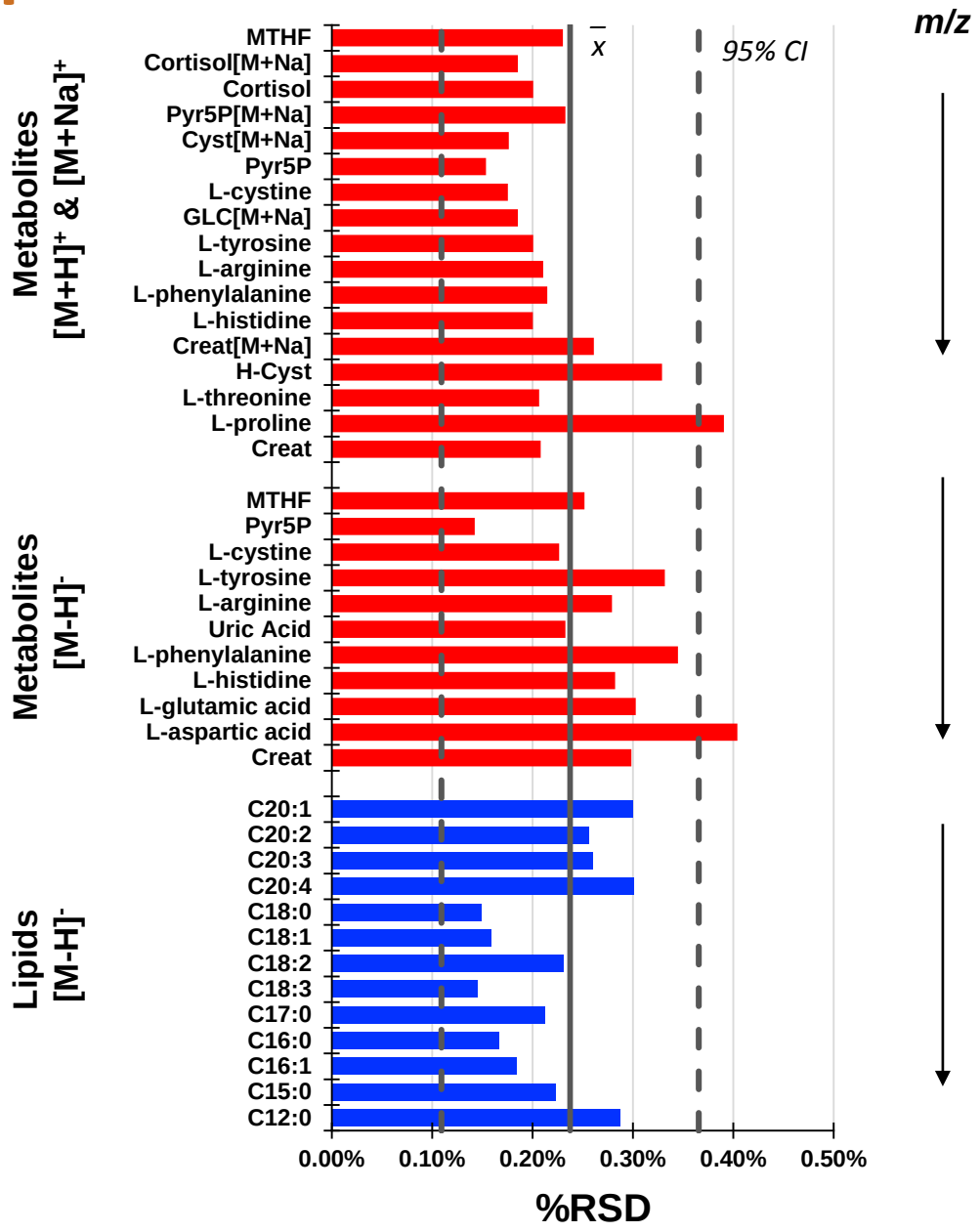


Pacific Northwest National Laboratory, Richland
Agilent Technologies, Inc., Santa Clara
Vanderbilt University, Nashville
BOKU – Univ. of Natural Resources and Life Sciences, Vienna

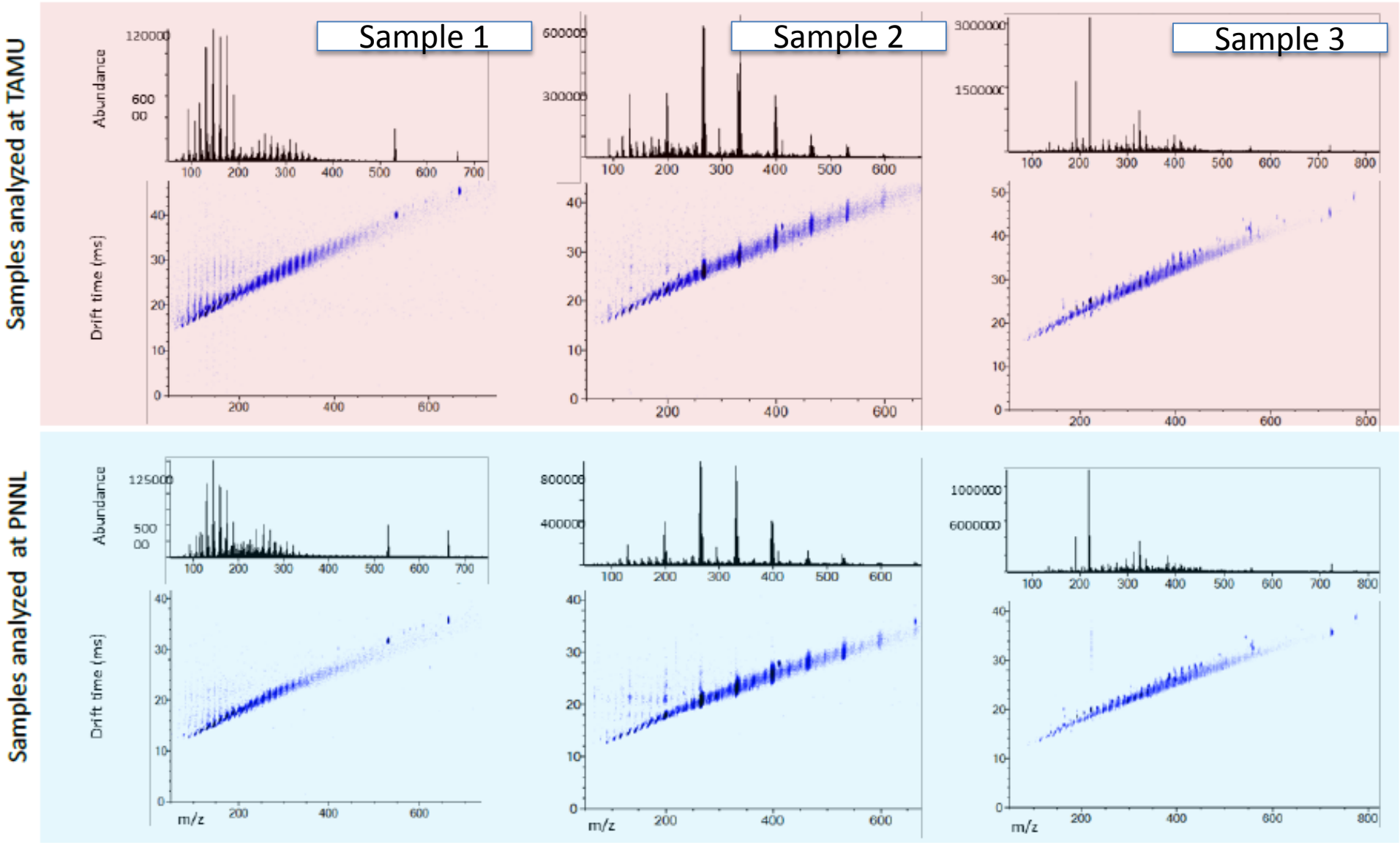
S. M. Stow, et al., *Anal. Chem.* **2017**, 89, 9048-9055.

Interlab precision comparison

- Ran triplicate injections at all 4 labs
- Analyzed in positive and negative ion mode
- Mean %RSD of 0.24%

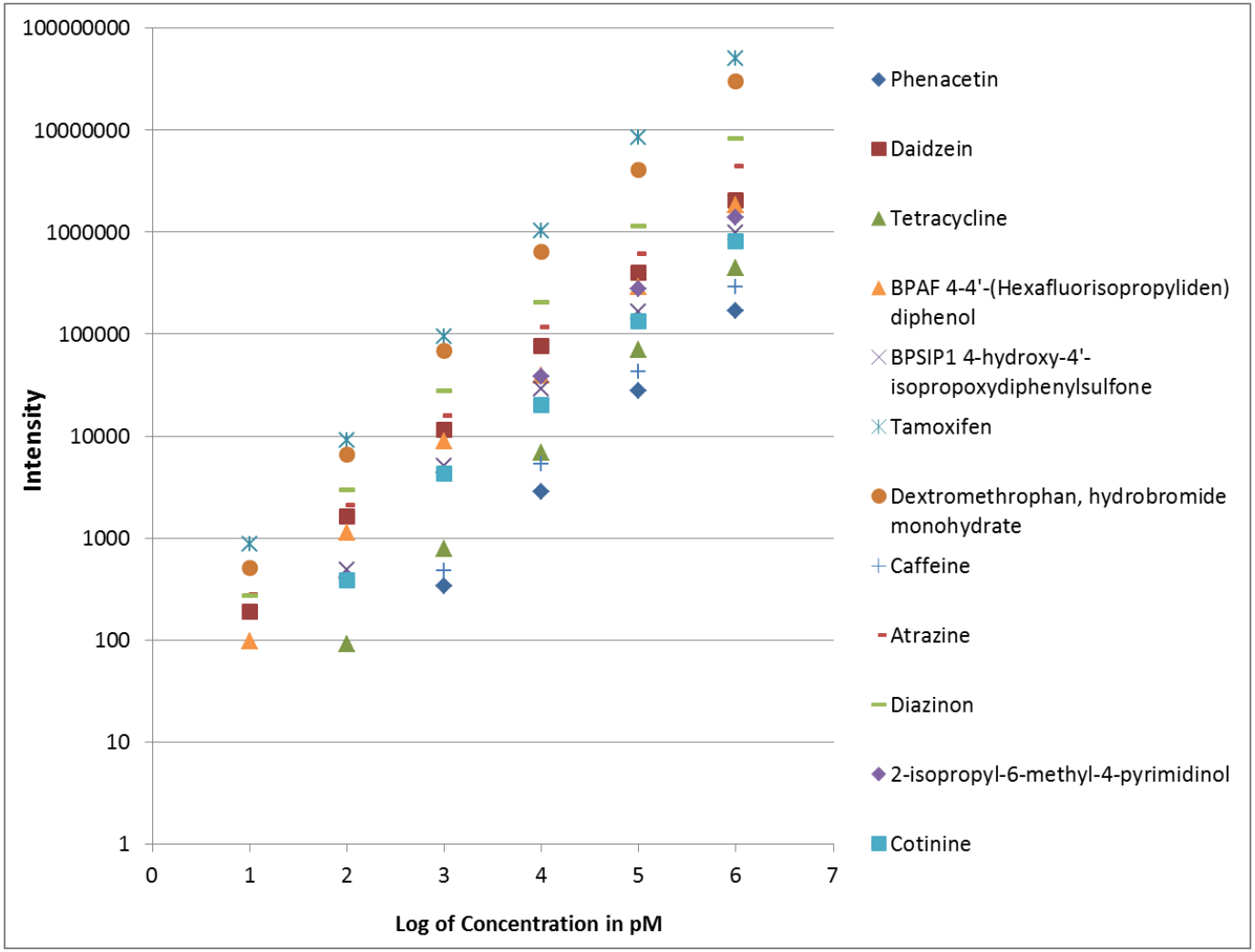


Interlab mixture reproducibility



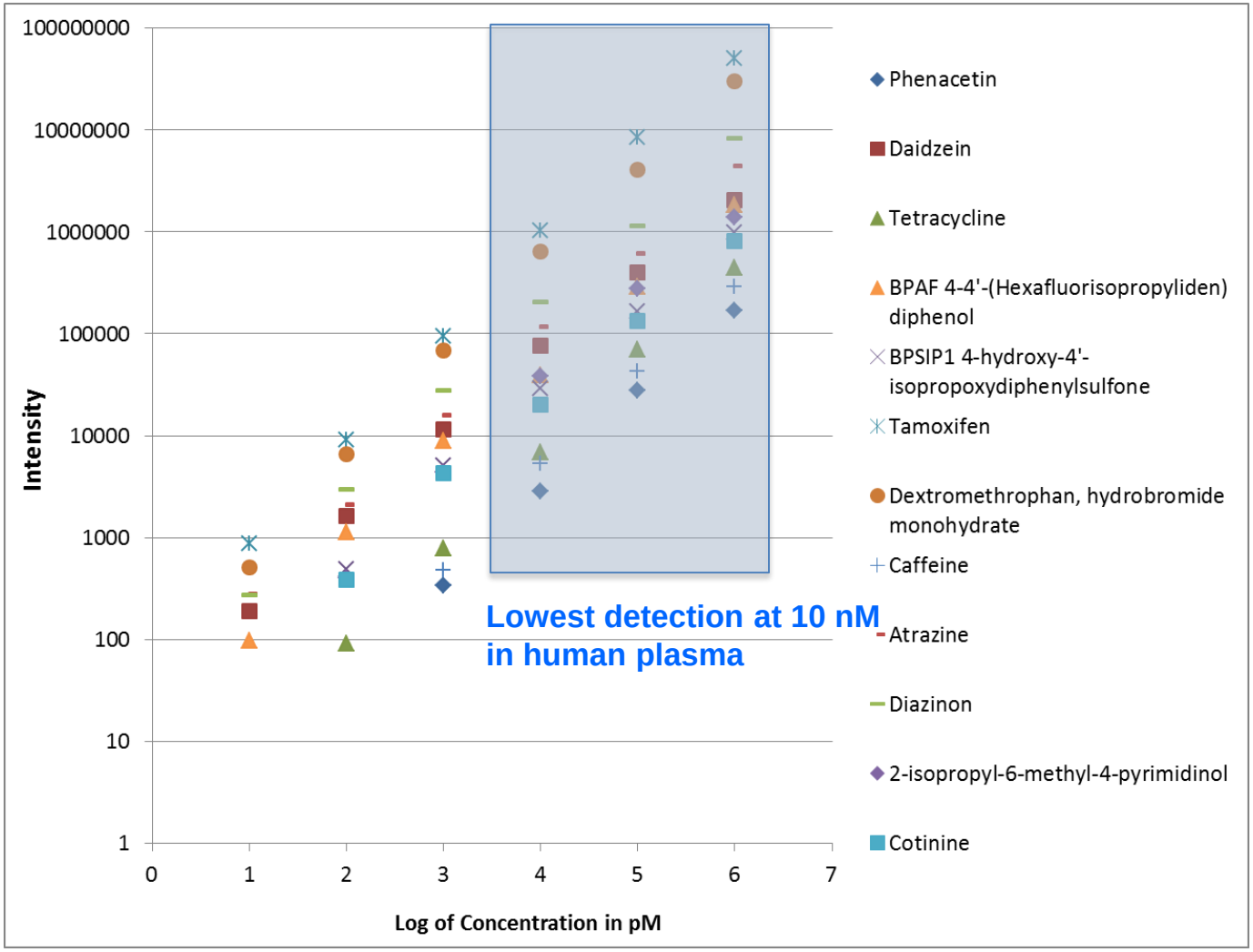
Collaboration with Ivan Rusyn's Group

Limit of detection in water using IMS-MS



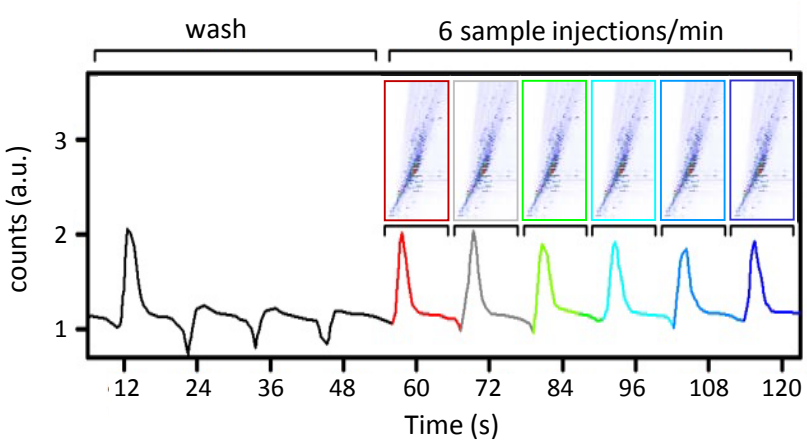
Lowest detection at 10 pM for standards in water
Linear concentration response

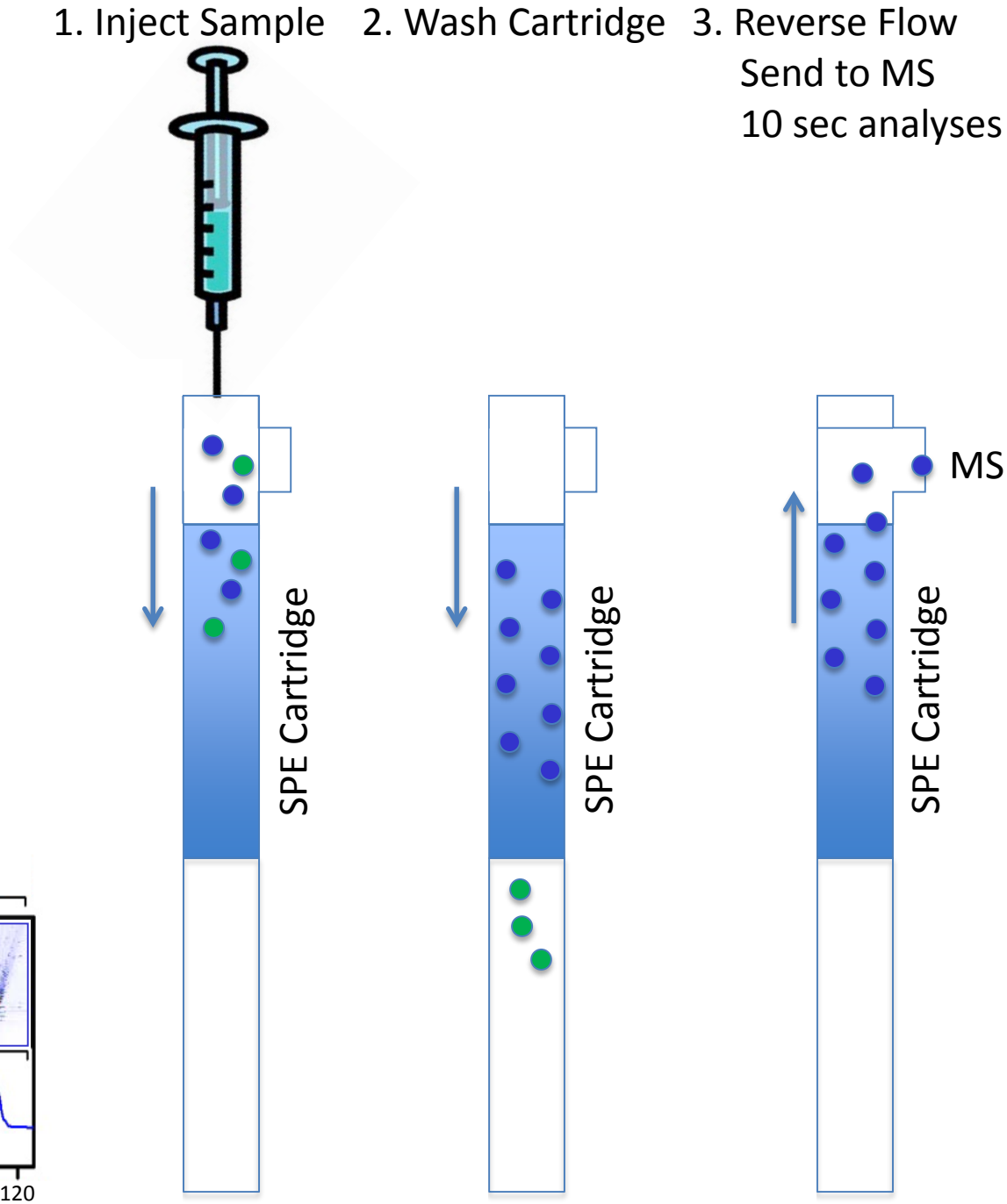
Limit of detection in human plasma with IMS-MS



Lowest detection at 10 pM for standards in water
Linear concentration response

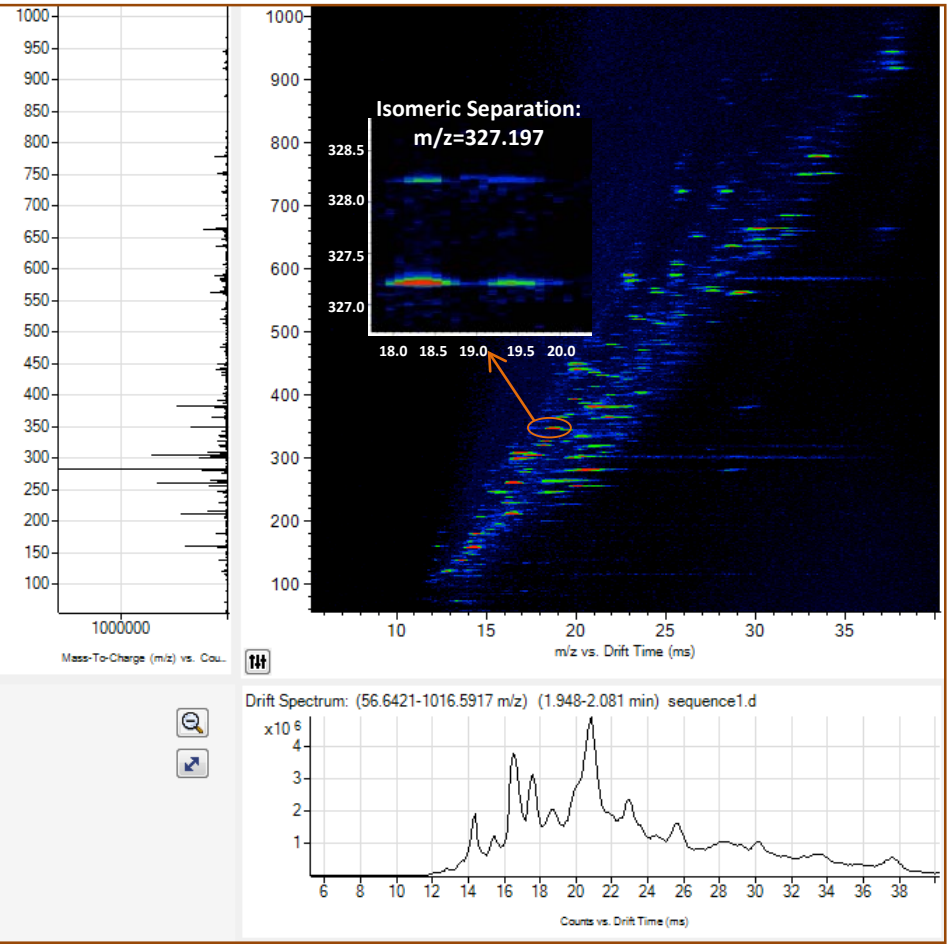
Automated SPE system





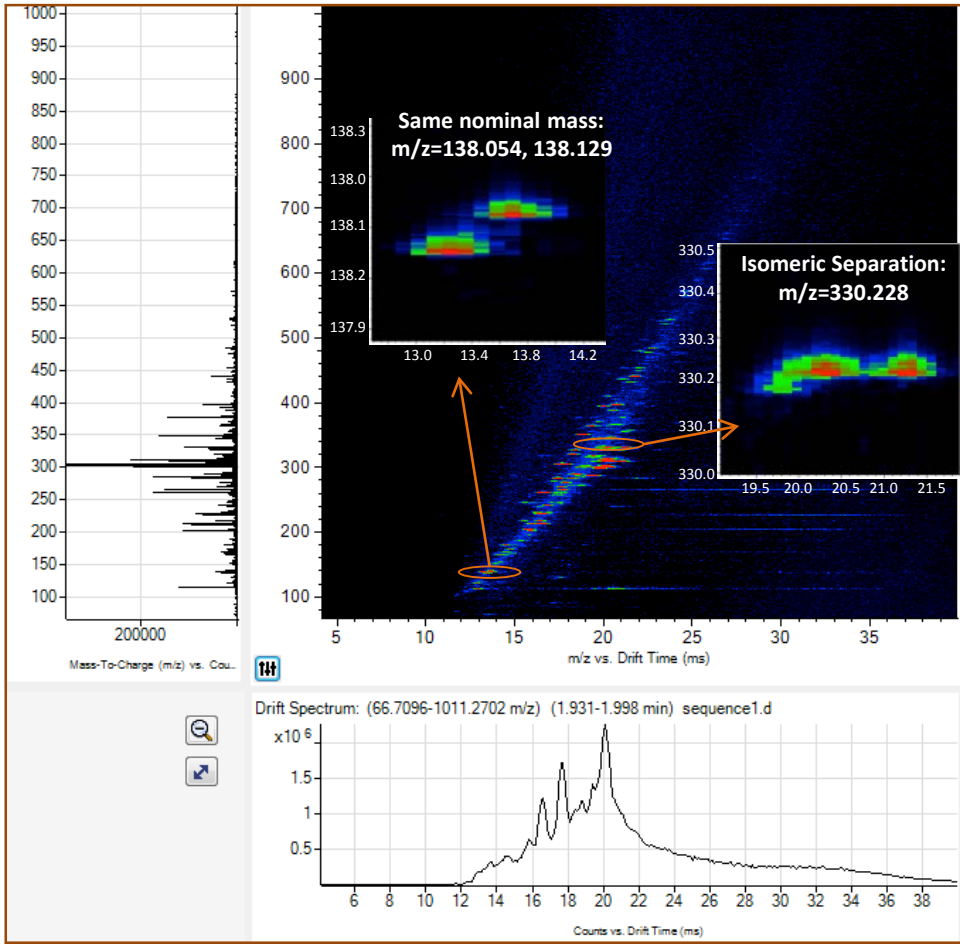
SPE-IMS-MS analyses of biological samples

Polar molecules extracted from mouse plasma



~1400 features with S/N > 5

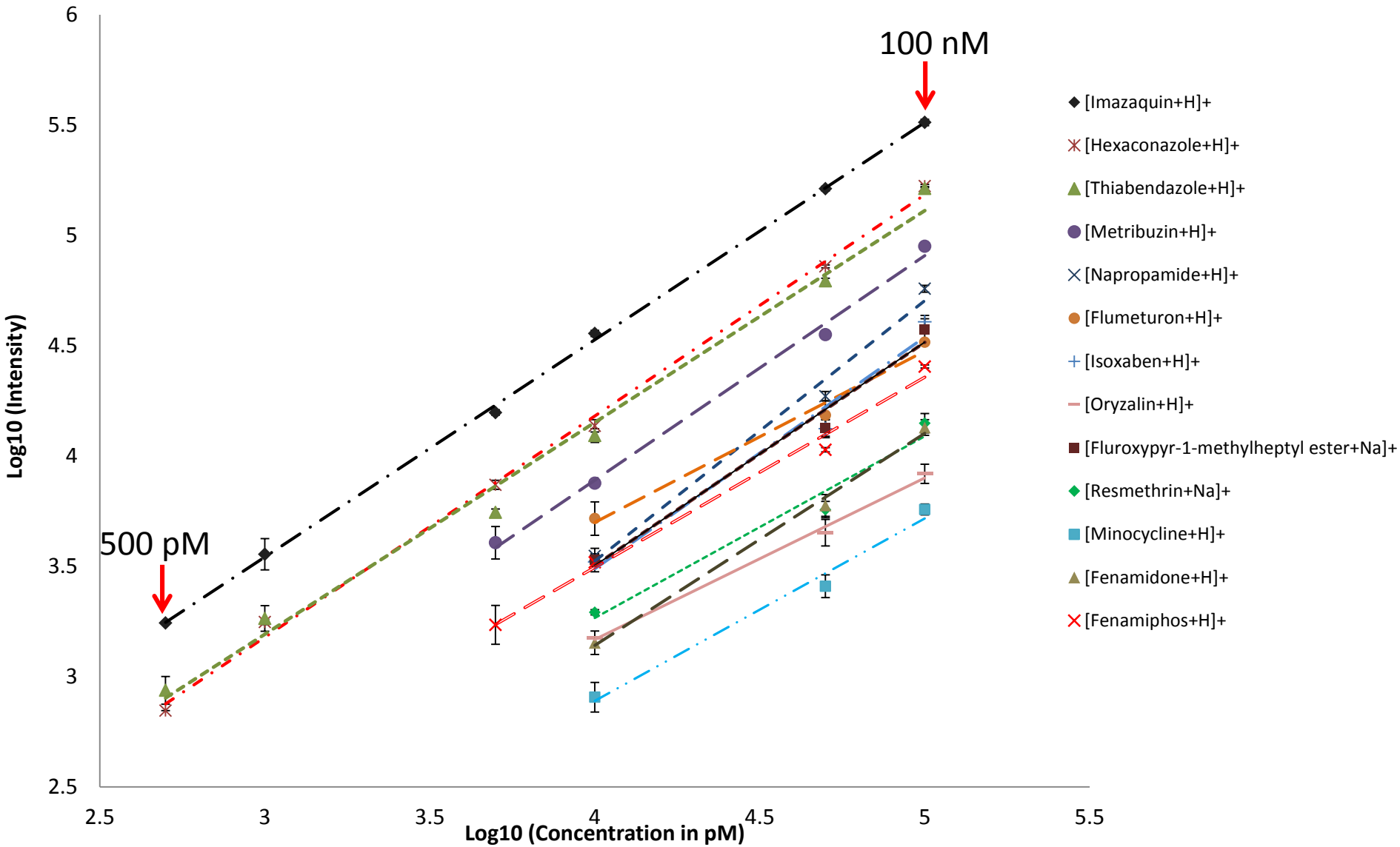
Polar molecules extracted from human urine



~1000 features with S/N > 5

X. Zhang, et al., *Clin. Mass Spectrom.* **2016**, 2, 1-10.

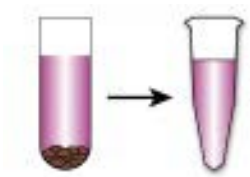
Calibration curve for xenobiotics in plasma



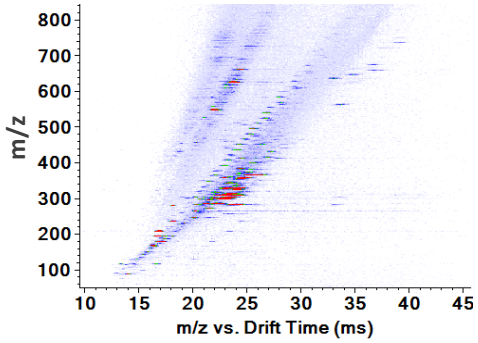
X. Zhang, et al., *Clin. Mass Spectrom.* **2016**, 2, 1-10.

Small molecule pipeline

Extraction



Instrumental Analysis



Data Processing & Analysis

ID	Mass	Intensity
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----	----	----
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----	----	----
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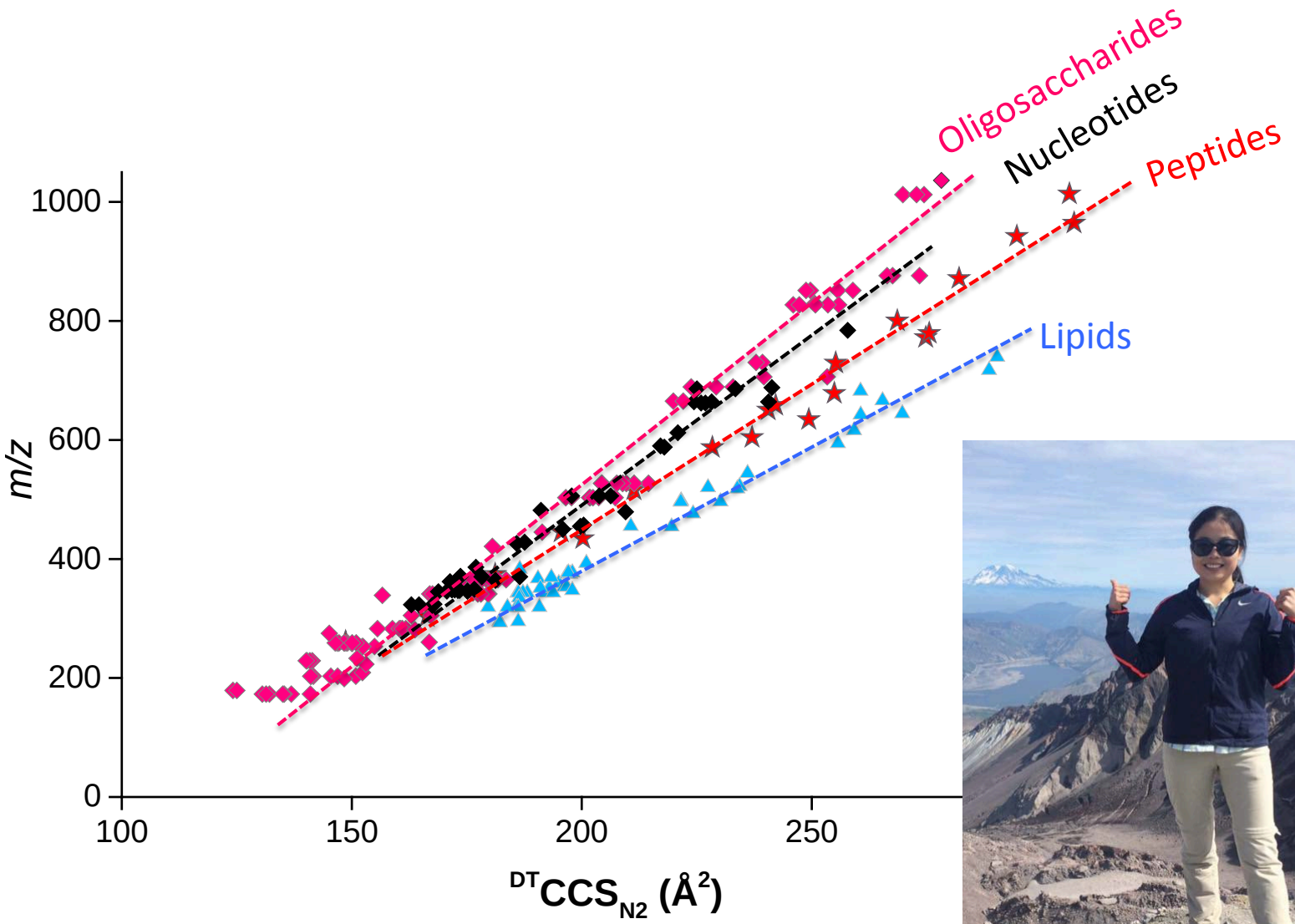
Methanol/Chloroform Extraction
(100 samples/day)

SPE-IMS-MS

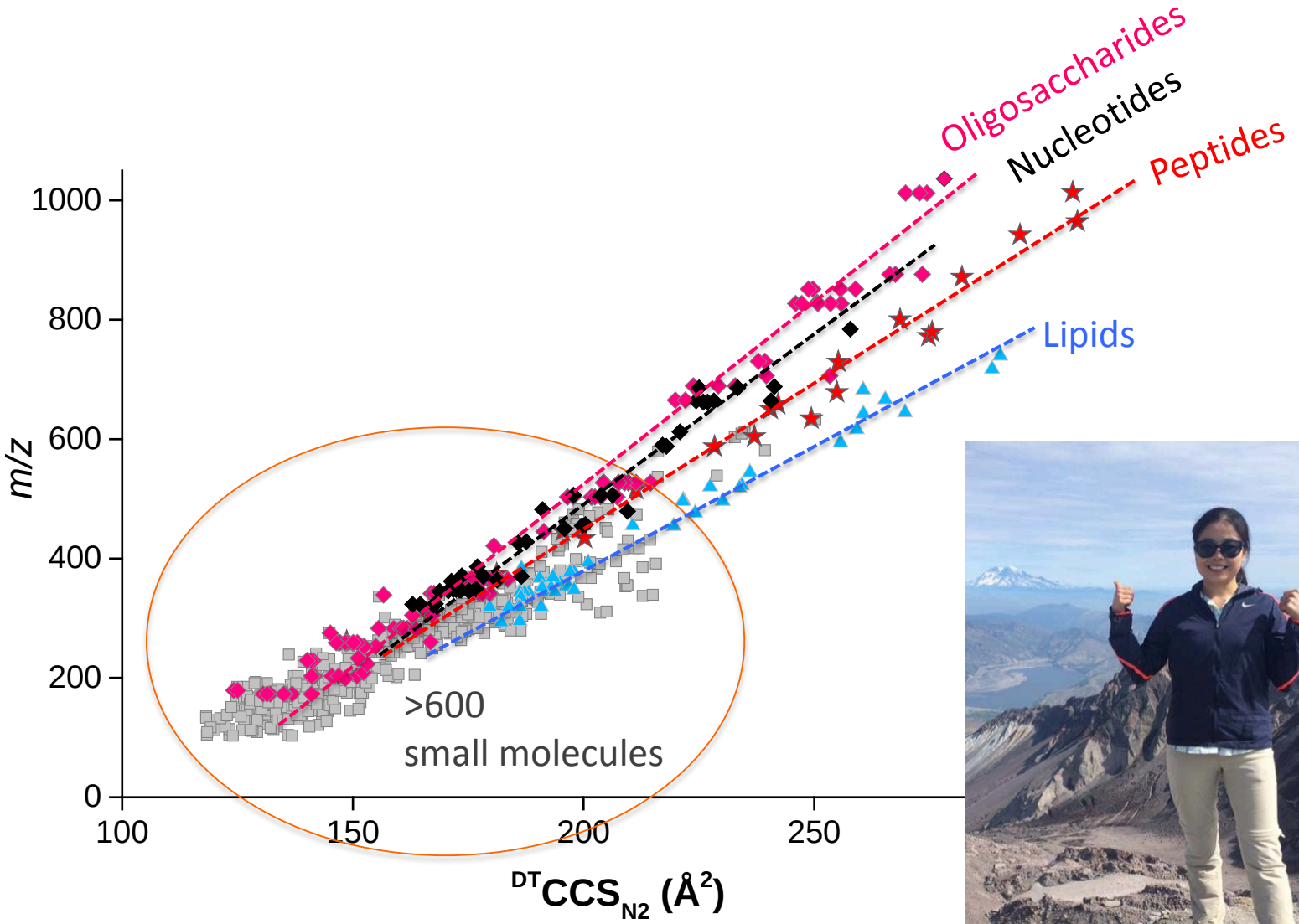
(8200 samples/day)

Database Matching & False Discovery Assessment
(? days)

Collision cross section database

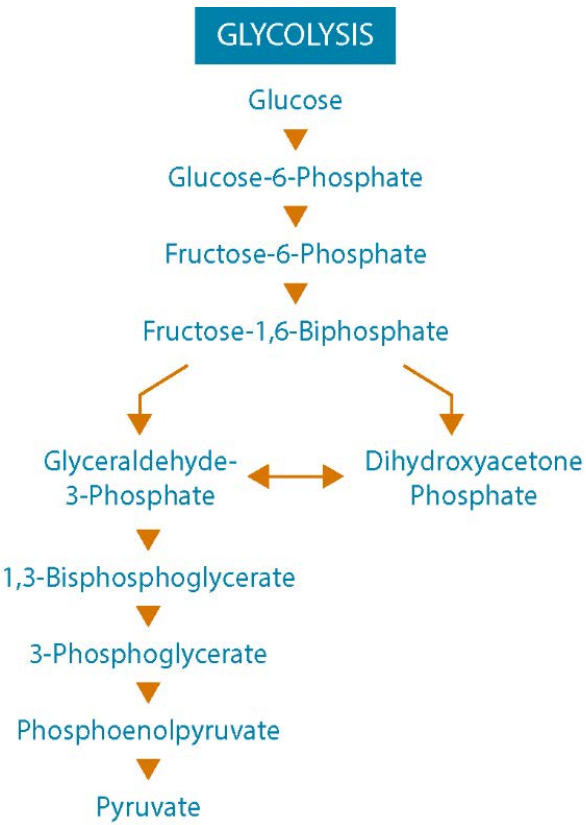


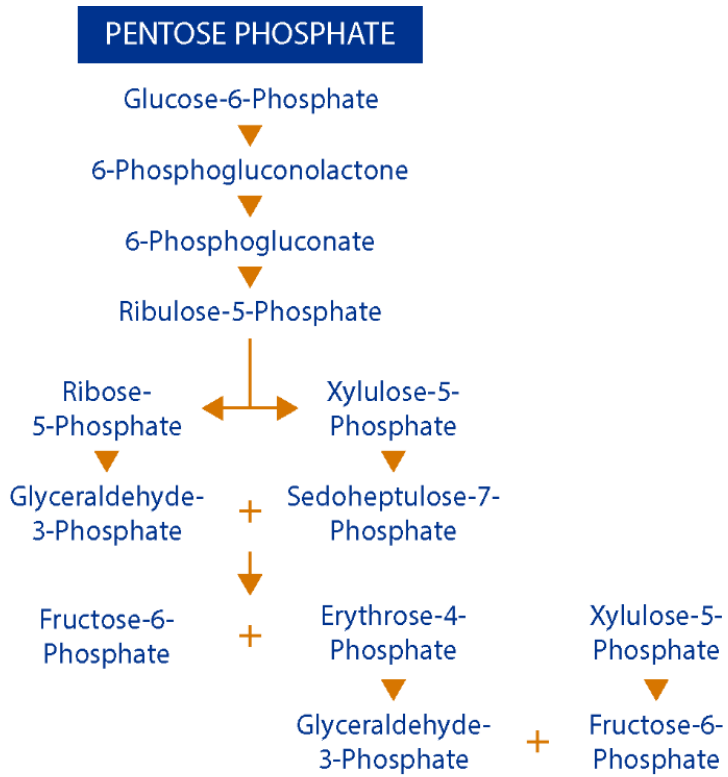
Collision cross section database

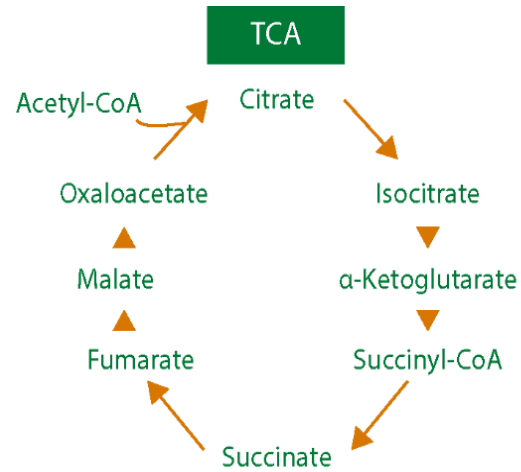


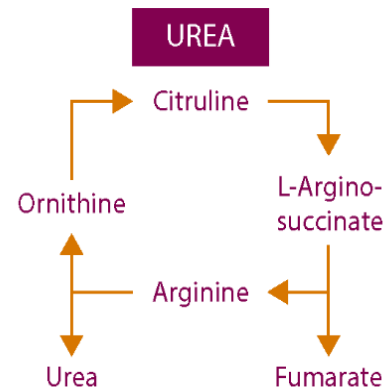
X. Zheng, et al., *Chem. Sci.* **2017**, ASAP.

Metabolic pathways



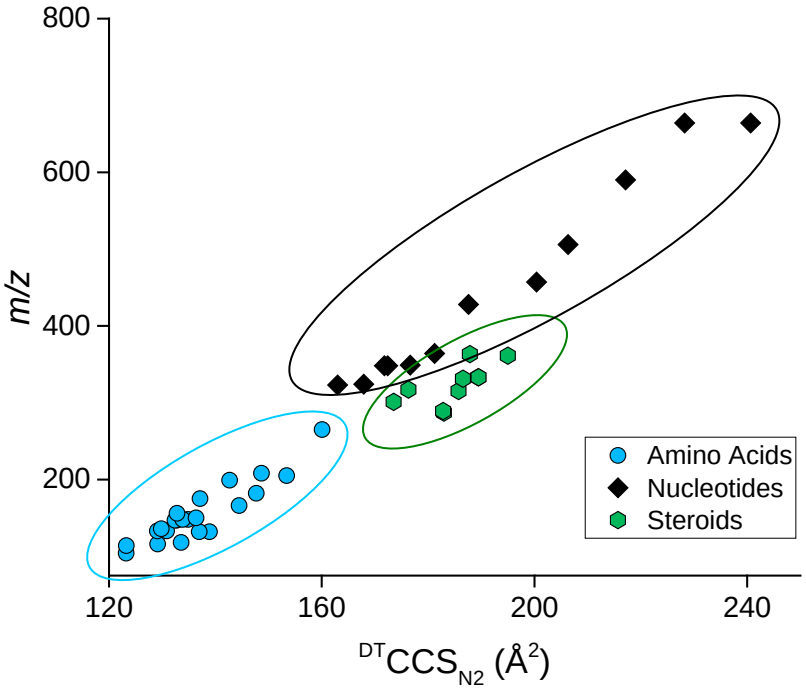






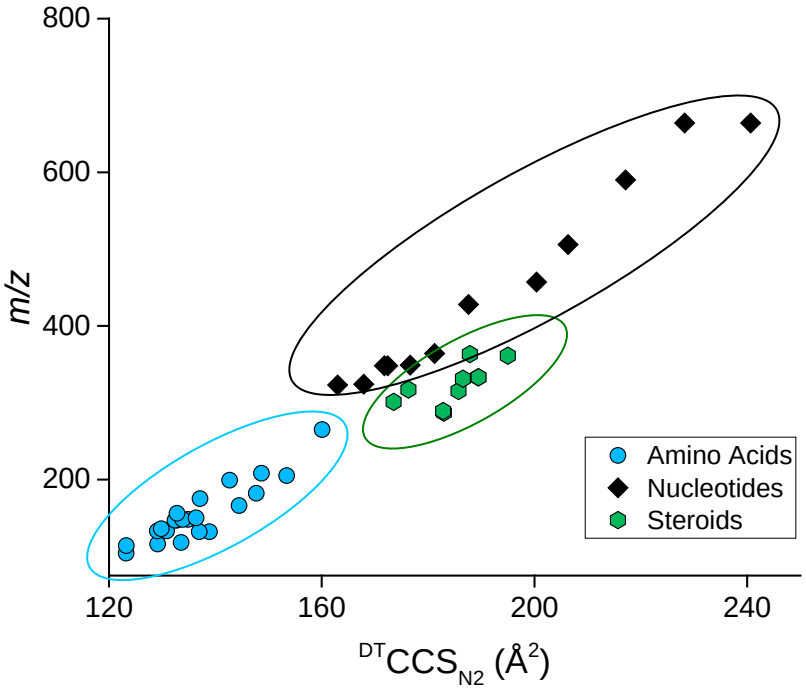
Primary metabolite trend lines

Protonated form

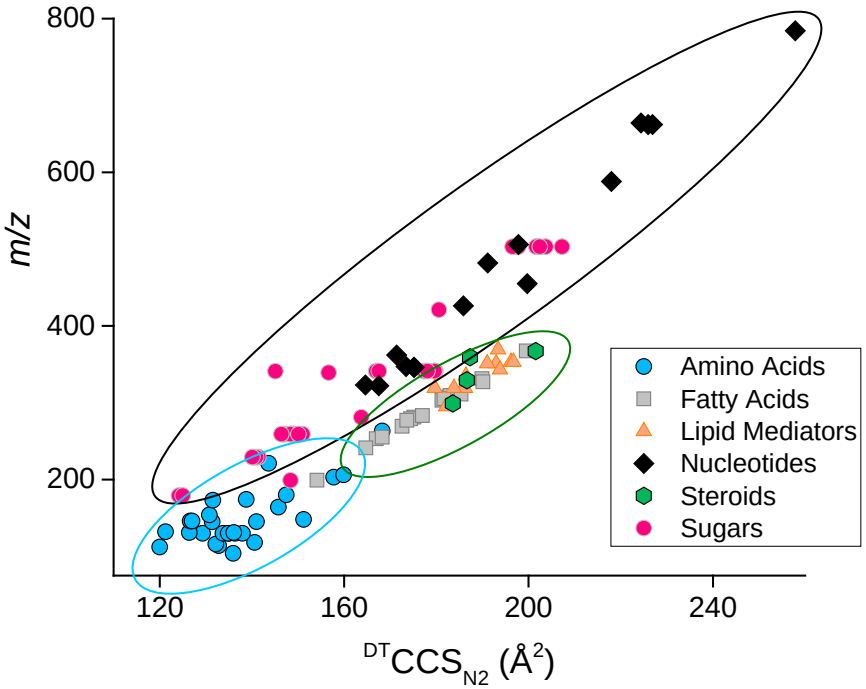


Primary metabolite trend lines

Protonated form



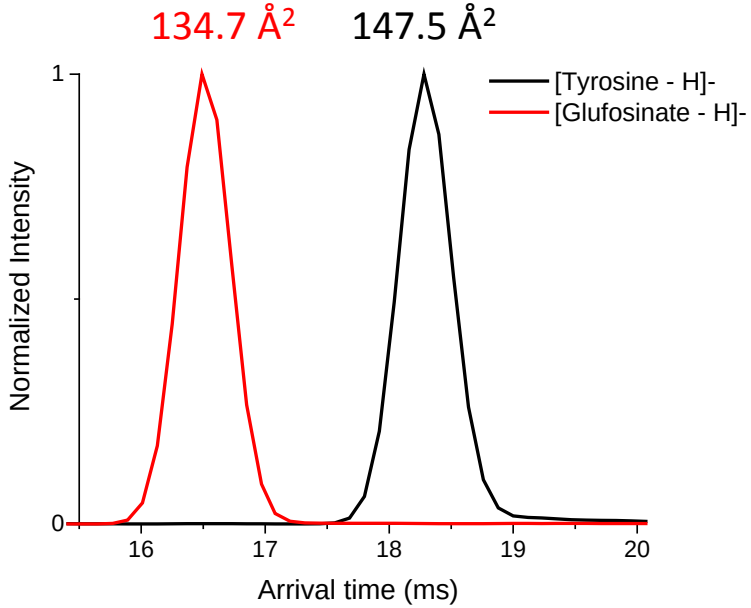
Deprotonated form



Primary metabolite versus xenobiotic

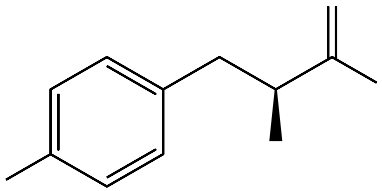
L-Tyrosine (amino acid)
Exact mass: 181.0738896

Glufosinate (herbicide)
Exact mass: 181.0503922

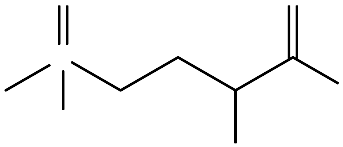


Deprotonated form

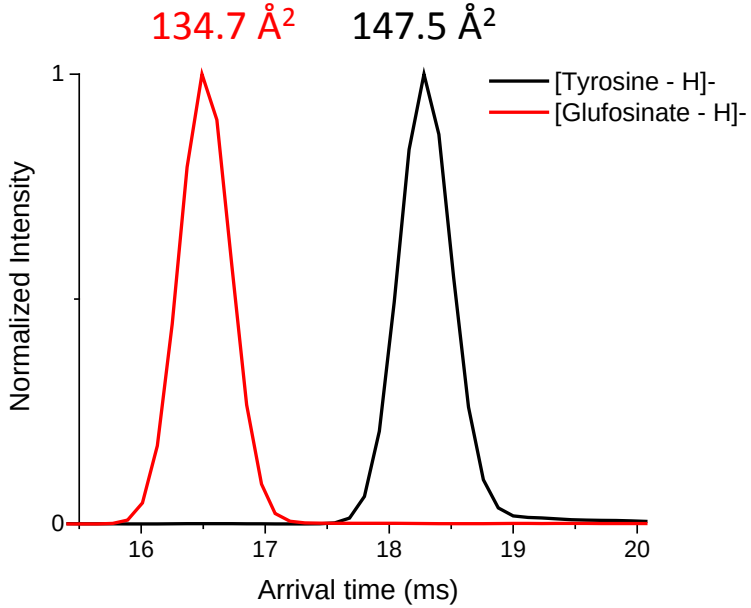
Primary metabolite versus xenobiotic



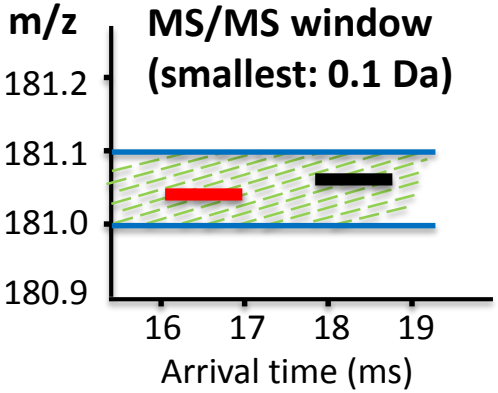
L-Tyrosine (amino acid)
Exact mass: 181.0738896



Glufosinate (herbicide)
Exact mass: 181.0503922



Deprotonated form



Website - http://panomics.pnnl.gov/metabolites/

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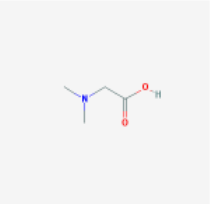
Home About Download Data ?

Results from manuscript "A Structural Examination and Collision Cross Section Database for Over 500 Metabolites and Xenobiotics Using Drift Tube Ion Mobility Spectrometry"

No Pathway Filter Show Pathway

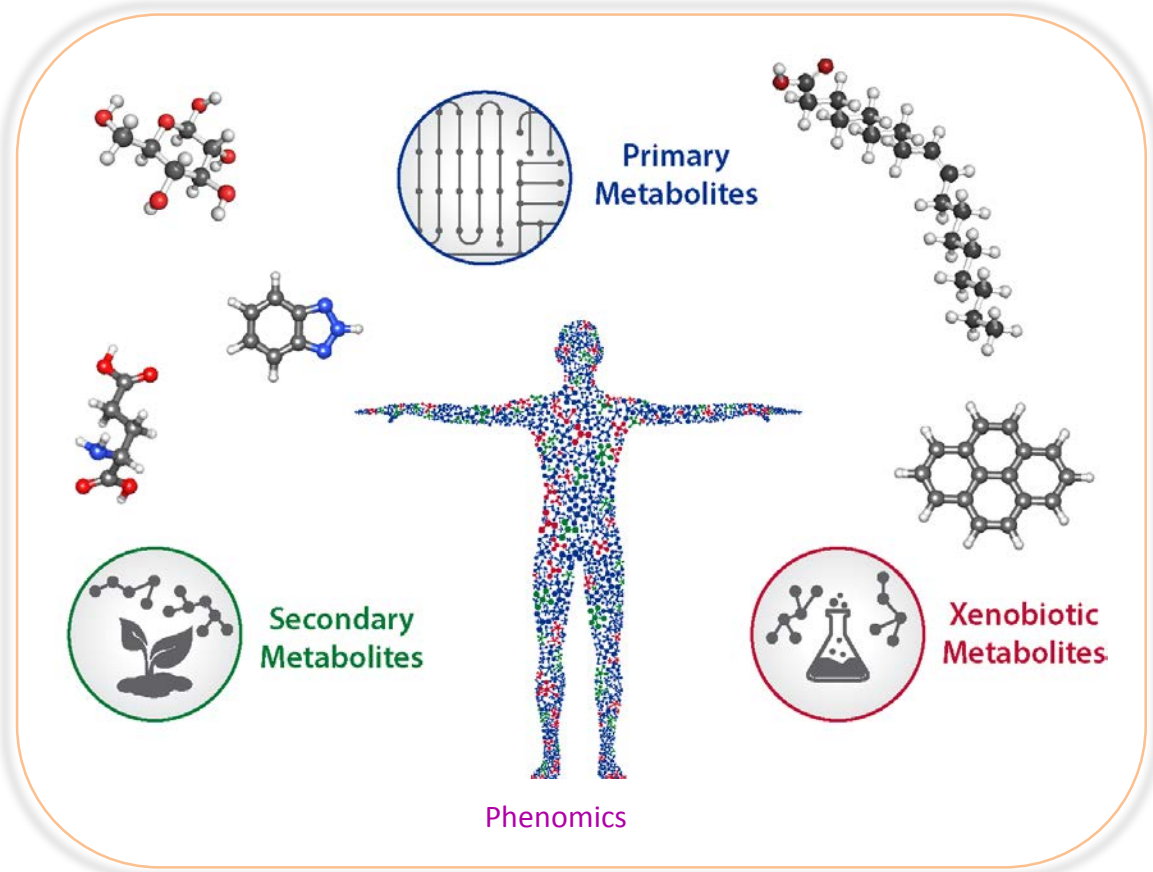
Primary Metabolite × Subclass Search:

Name	CAS Number	Formula	Structure	Exact Mass	$^{DT}CCS_{N_2}$ (Å^2)
Class Primary Metabolites					
Subclass Amino Acids					
N,N-Dimethylglycine	1118-68-9	C4H9NO2		103.120336	$[M+H]^+$: 123.90

X. Zheng, et al., *Chem. Sci.* **2017**.

Summary

- Ion mobility spectrometry enables rapid structural analyses
- Combining multiple separations and methods enables faster and better small molecule identifications (i.e. SPE-IMS-MS)



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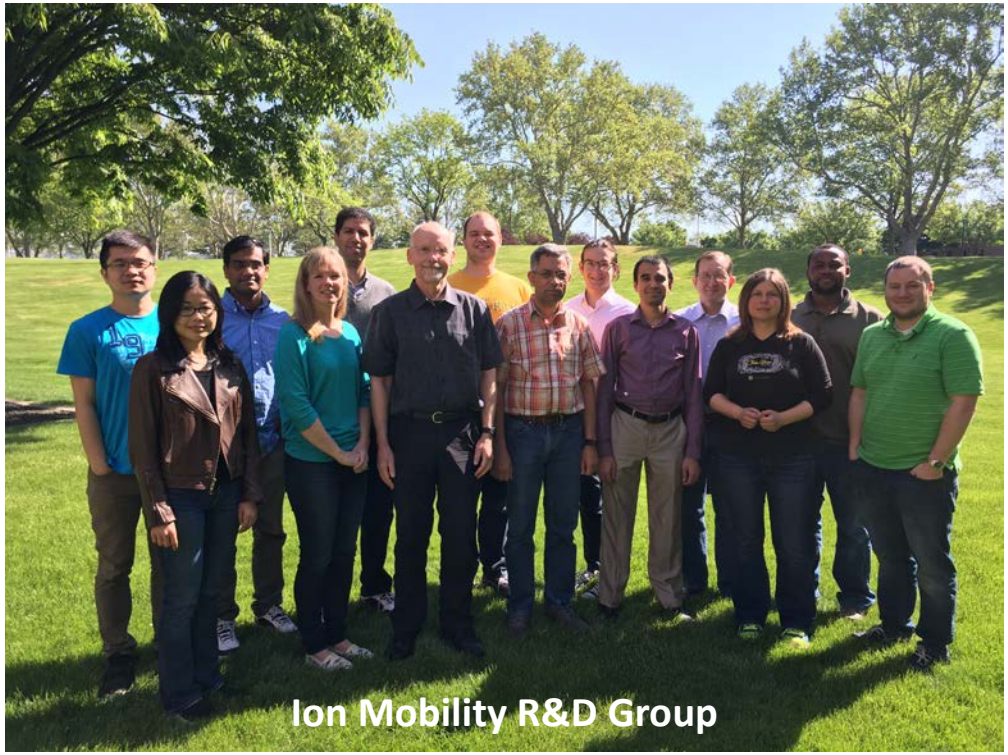

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