Pa ı i o
NATIONAL LABORATORY
Proudly Operated by Battelle *Since 1965*

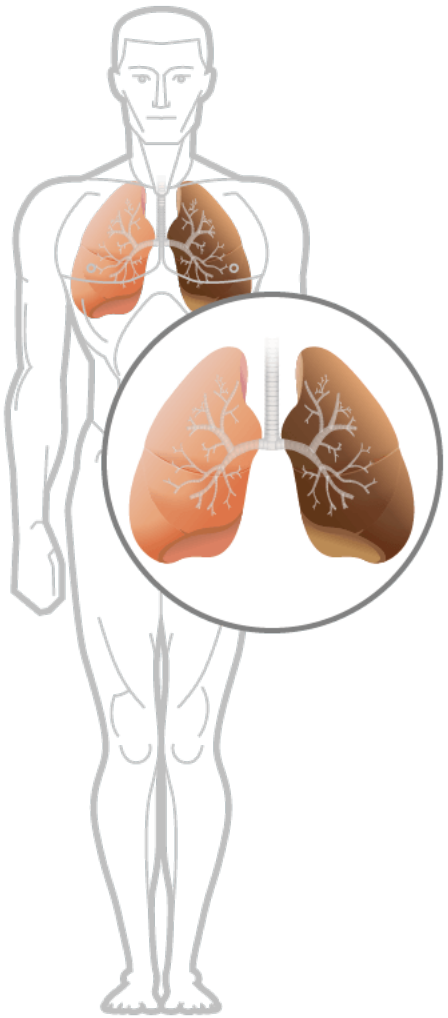
Rapid sample analyses for environmental toxicants

Erin Shammel Baker
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

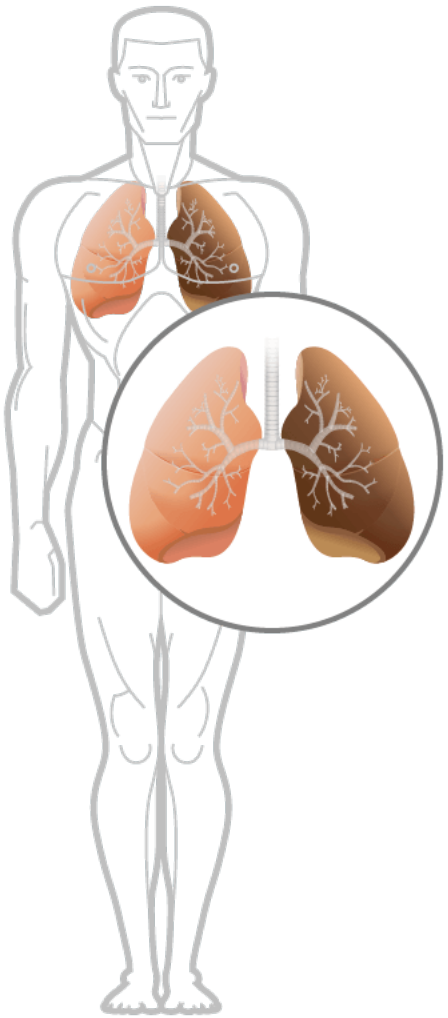
Understanding health risks

Health Risks

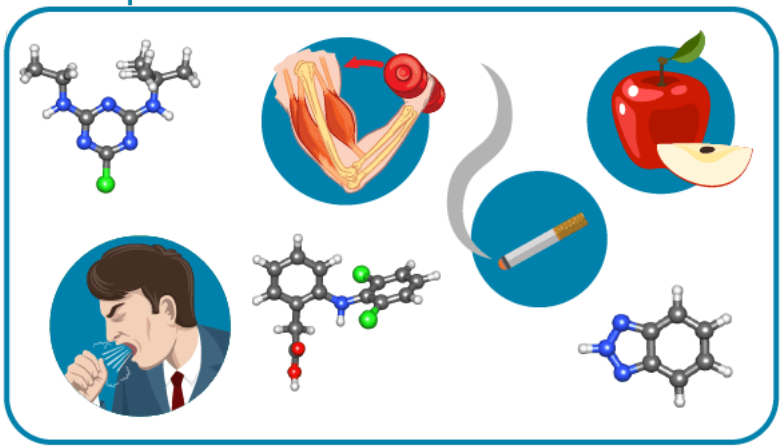


Understanding health risks

Health Risks

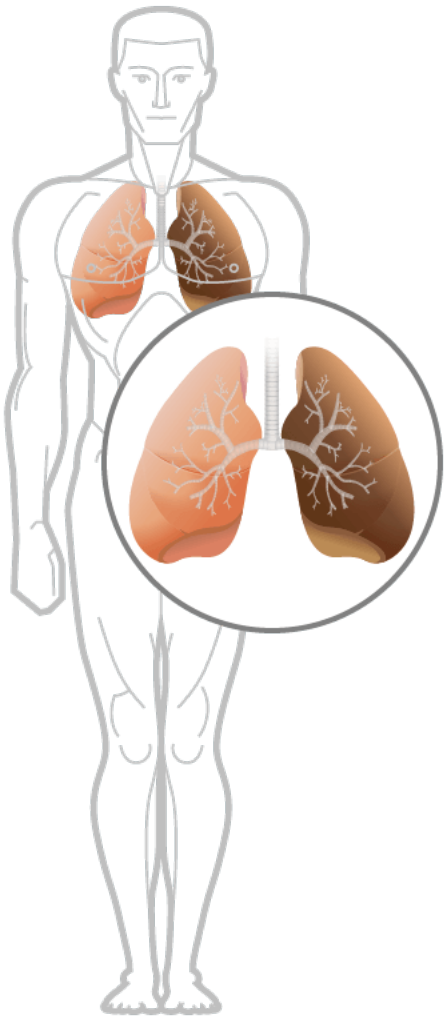


Specific External Environment

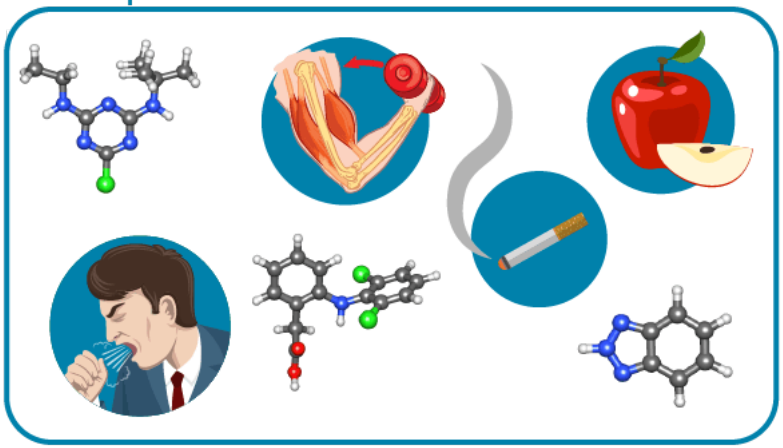


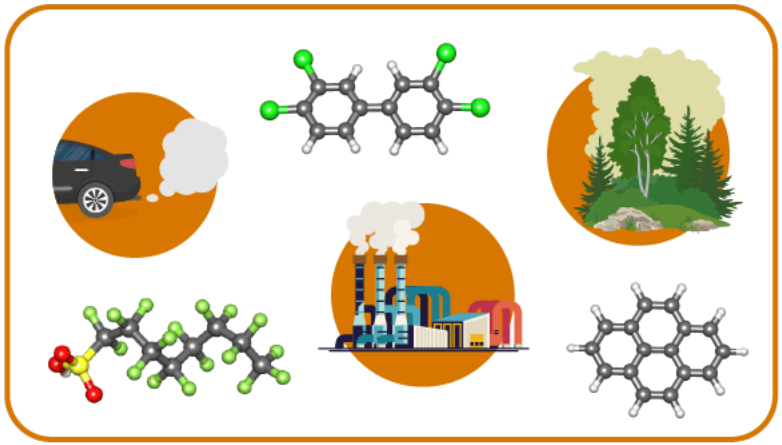
Understanding health risks

Health Risks



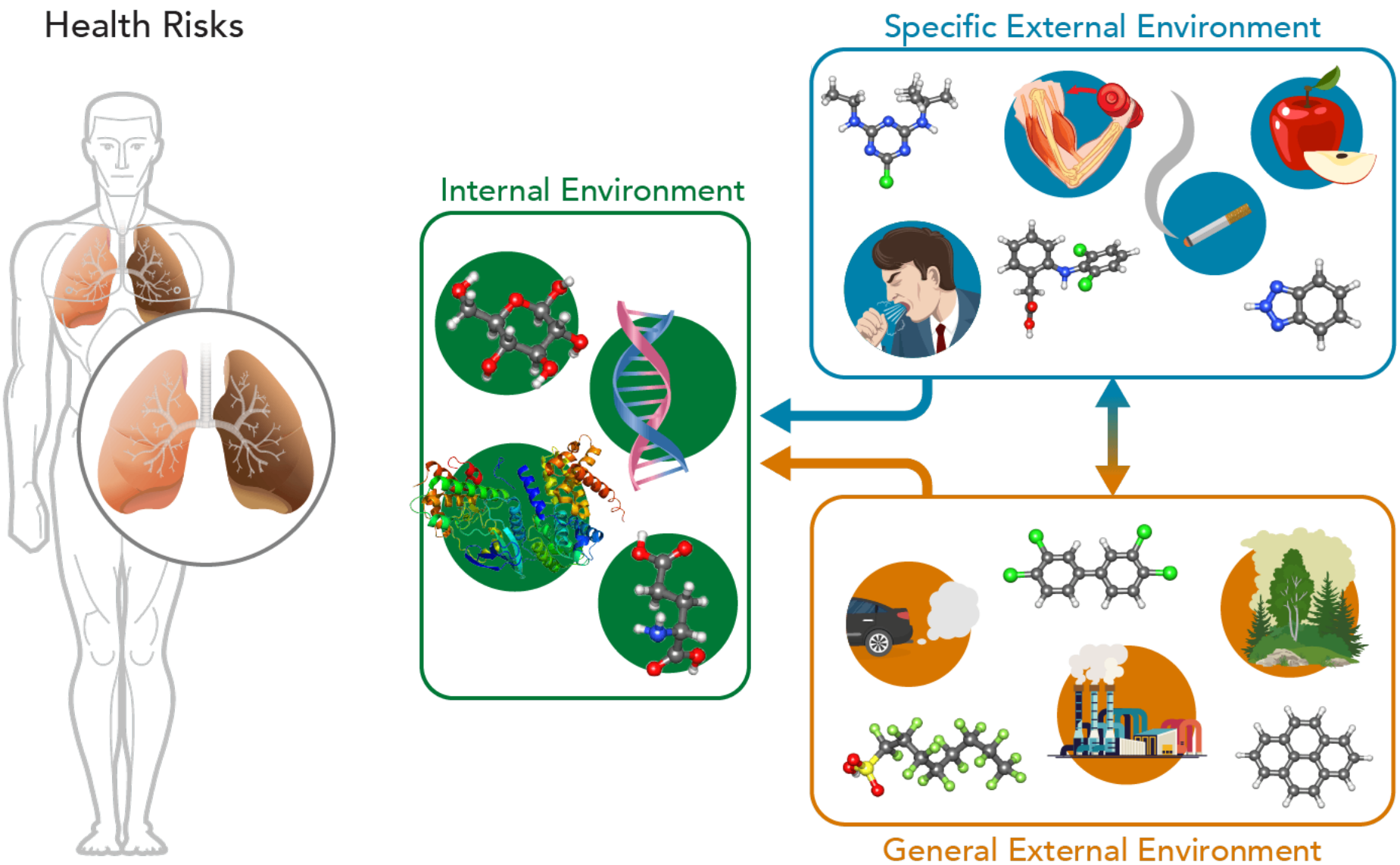
Specific External Environment



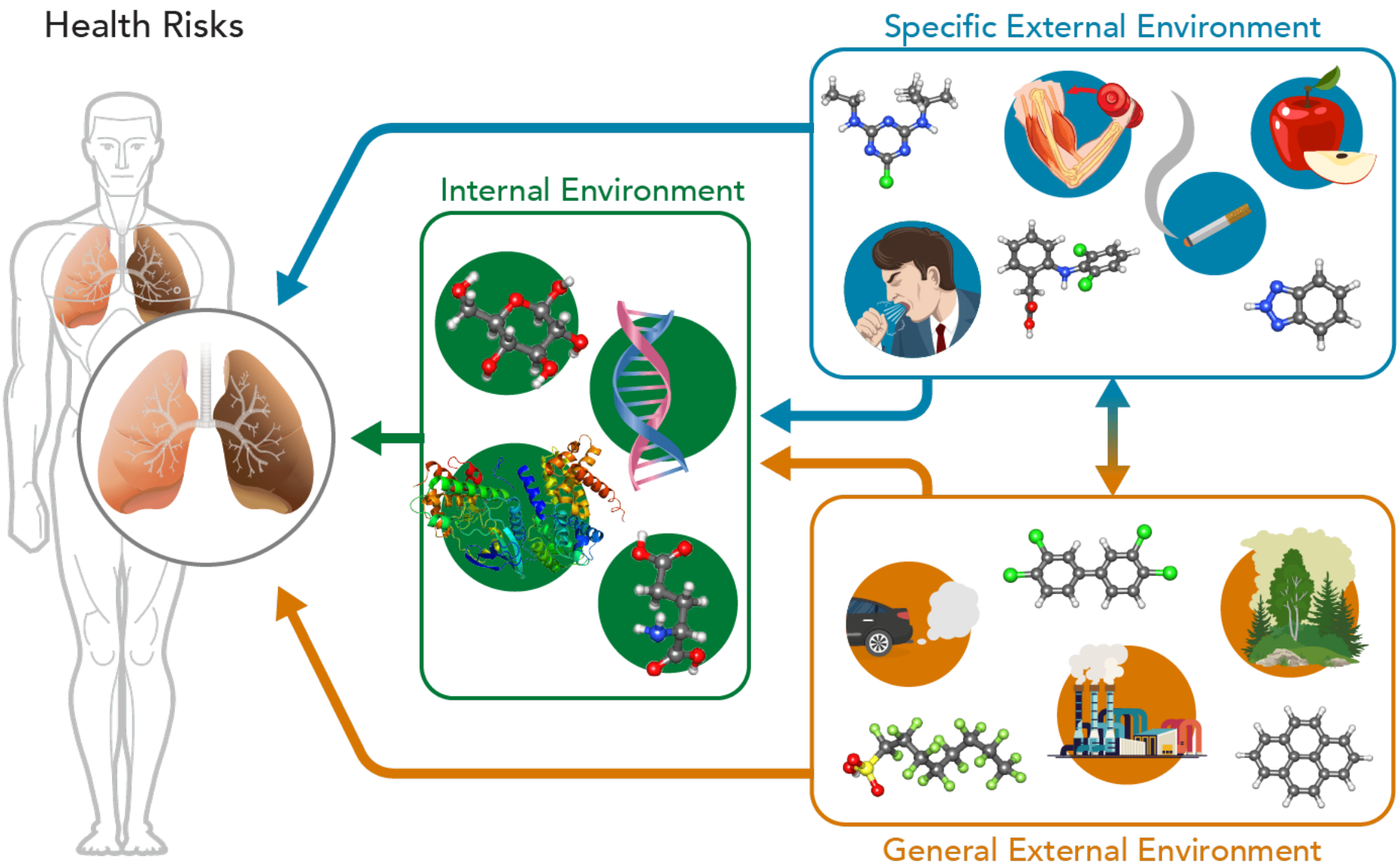


General External Environment

Understanding health risks

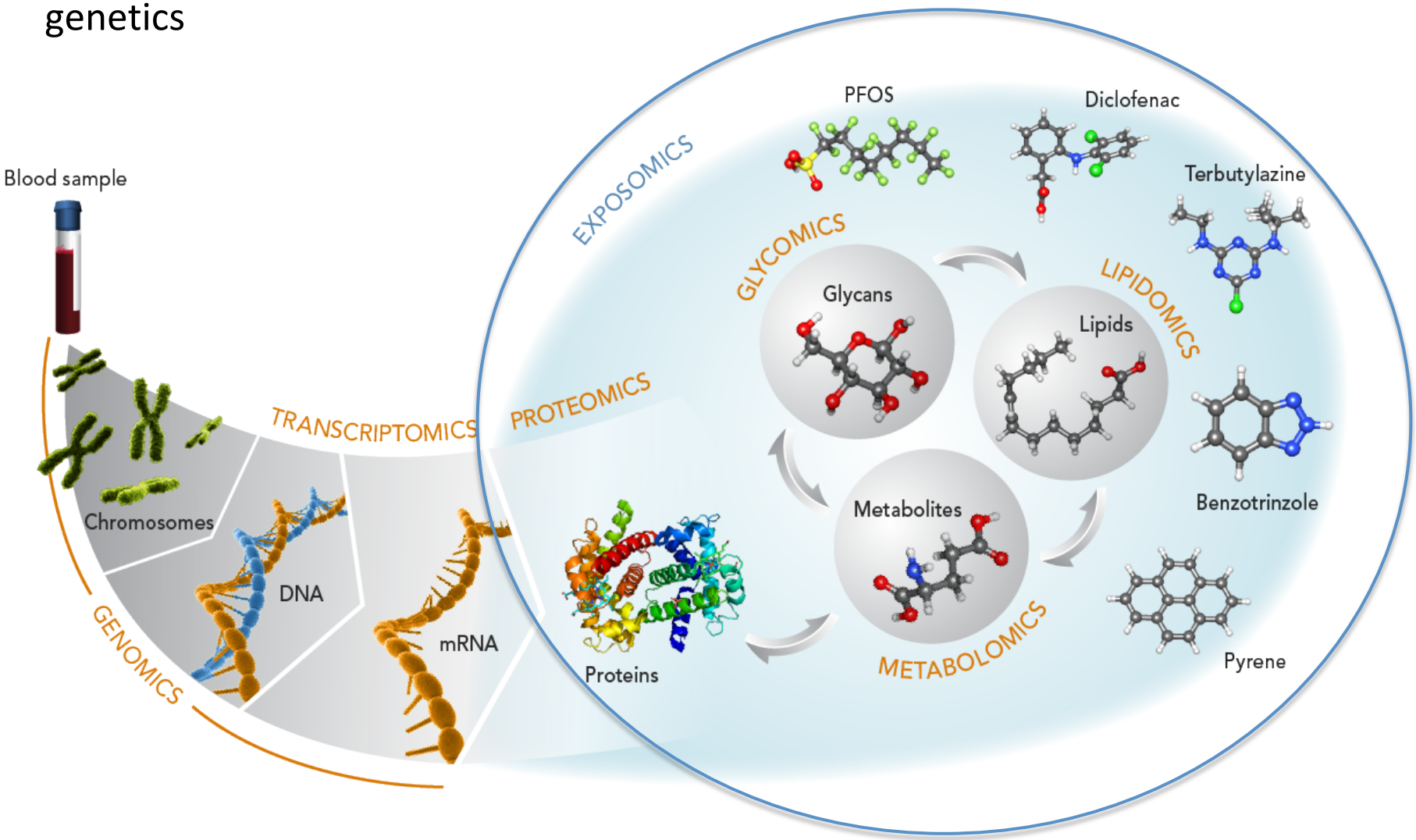


Understanding health risks



Multi-omic analyses

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



MS-based Measurements

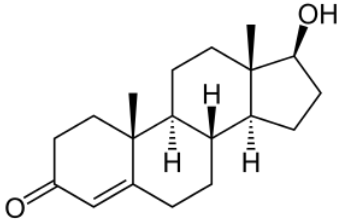
Main challenges with small molecule measurements

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

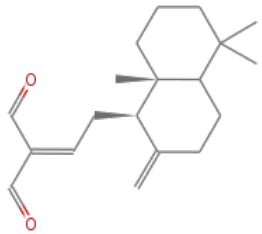
2. Biological changes are best understood when both endogenous metabolites and xenobiotics are analyzed

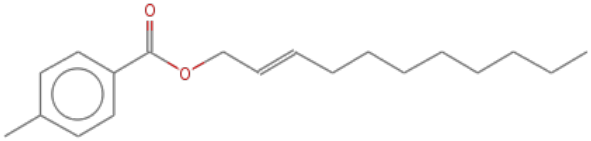
3. Untargeted measurements covering thousands of small molecules are desired for time course studies and large cohort analyses

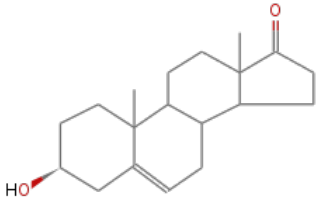
4. Many small molecules have the same masses but a different chemical makeup so distinguishing them with MS-based approaches can be difficult or impossible



Testosterone
Exact mass = 288.2089188

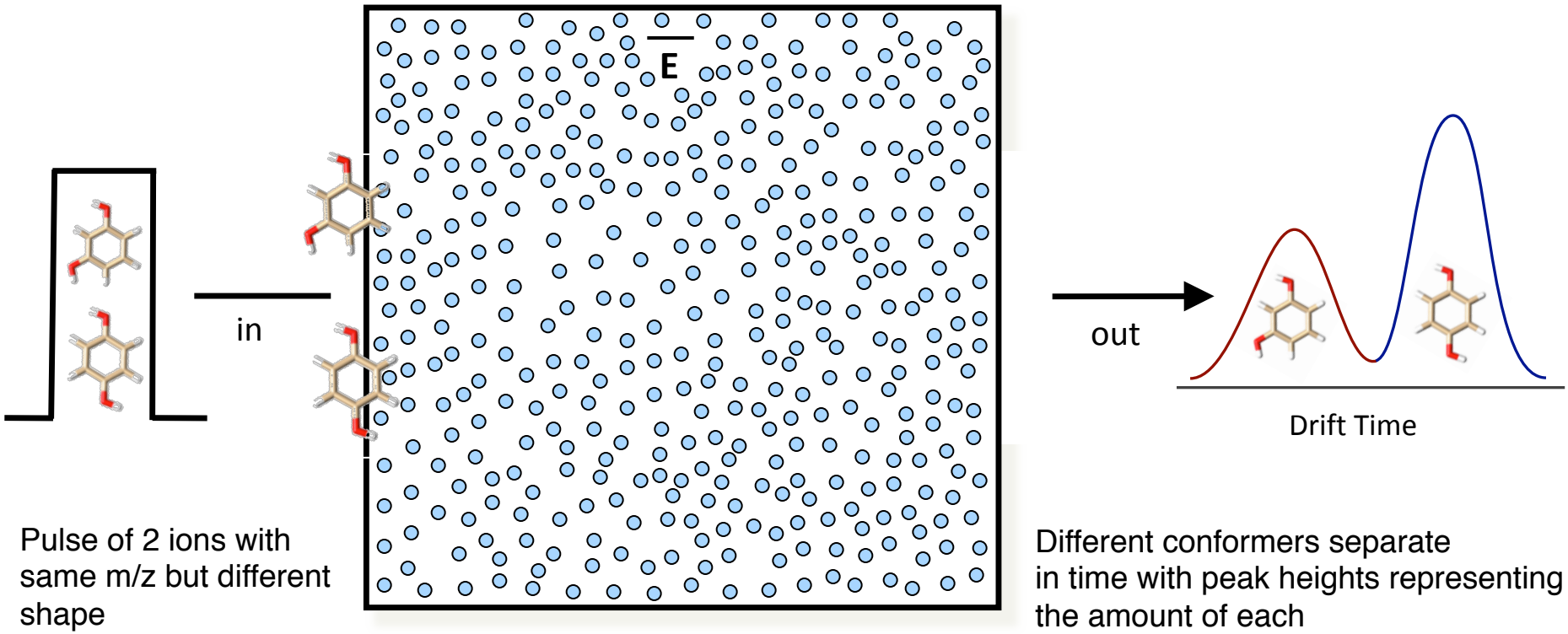






In the NIST database there are
18 different options with exact mass = 288.2089188

Ion mobility concept



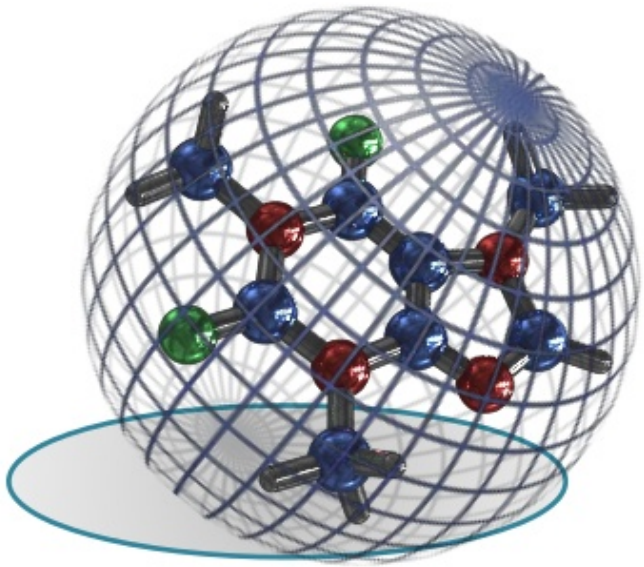
velocity is constant

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

K = ion mobility

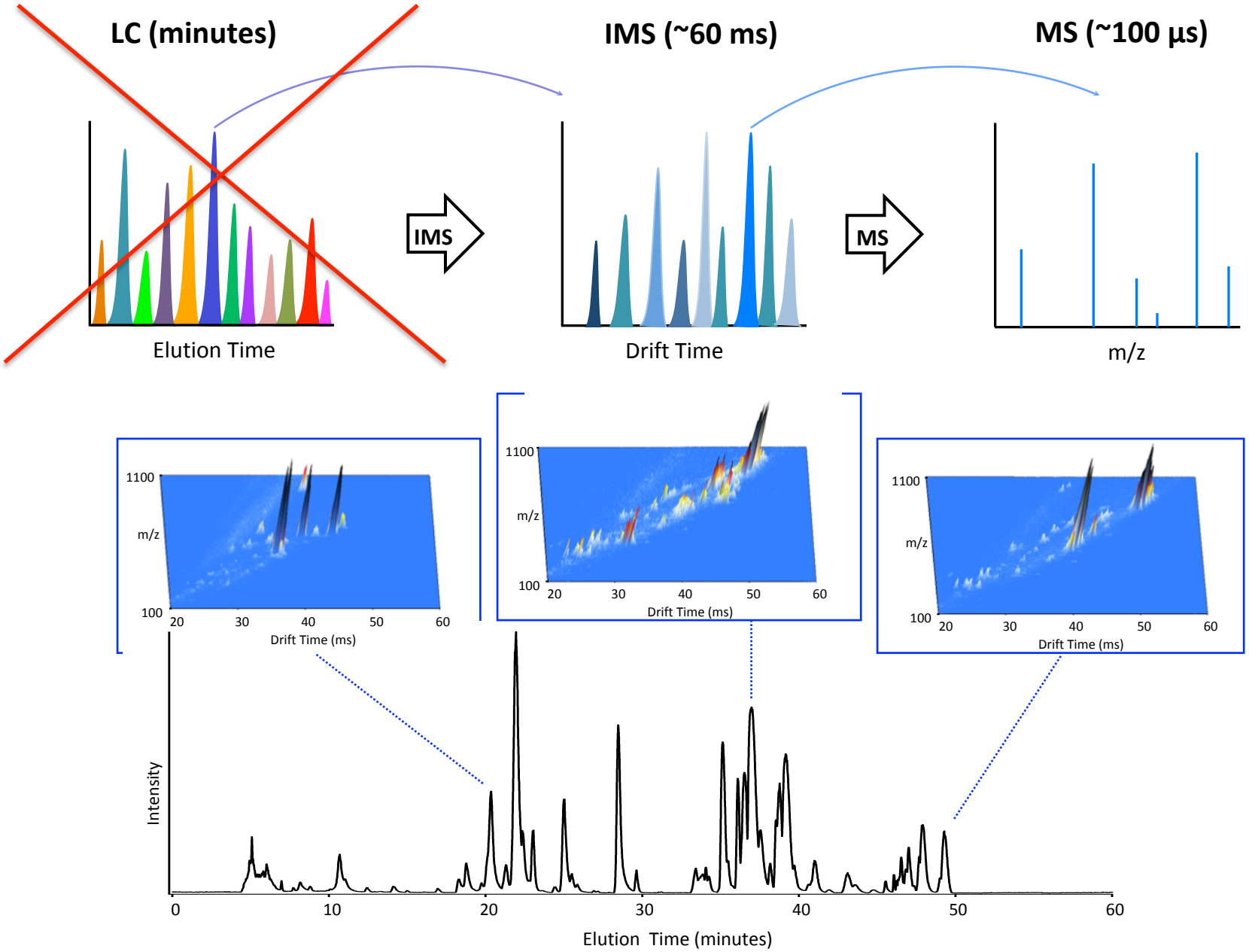
Ion-neutral collision cross section (CCS)

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

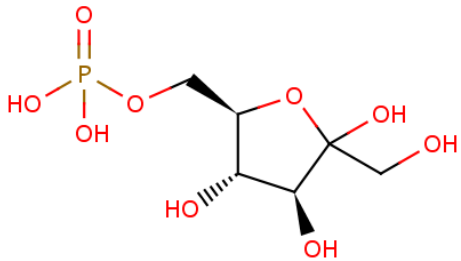


Slide courtesy of Professor Kevin Pagel and Waters

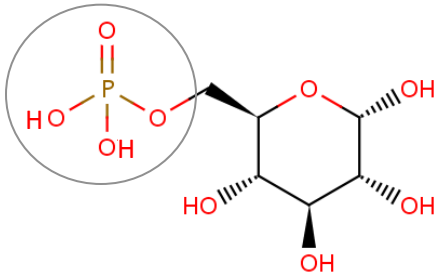
Ion mobility concept



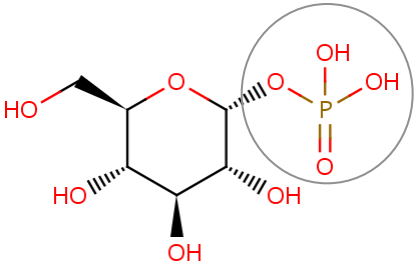
Isomers difficult to separate with hydrophobic interaction liquid chromatography (HILIC)



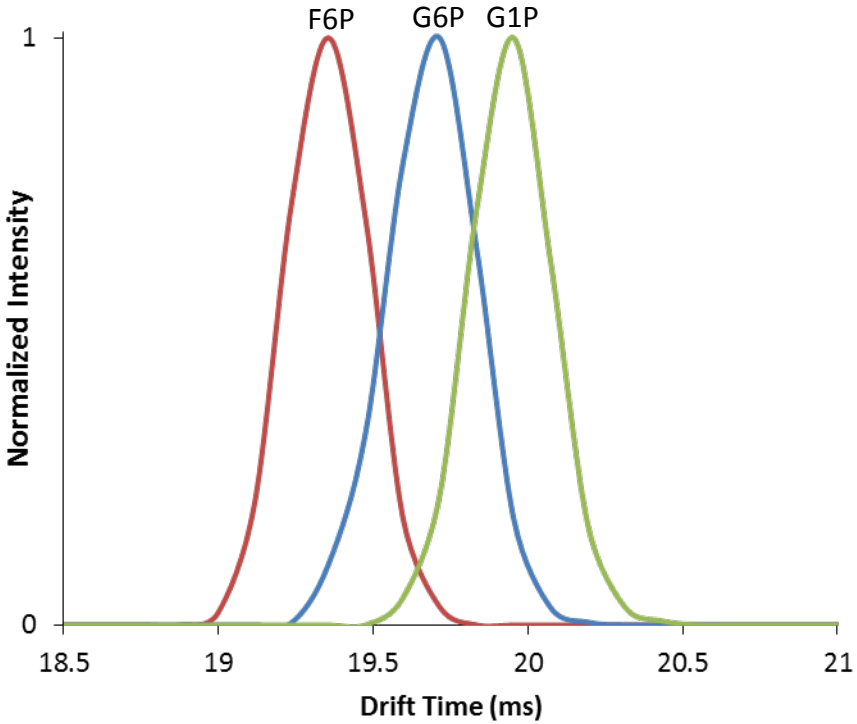
D-Fructose-6-phosphate (F6P)



D-Glucose-6-phosphate (G6P)

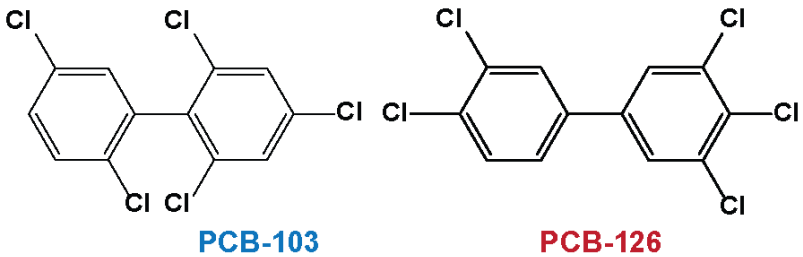


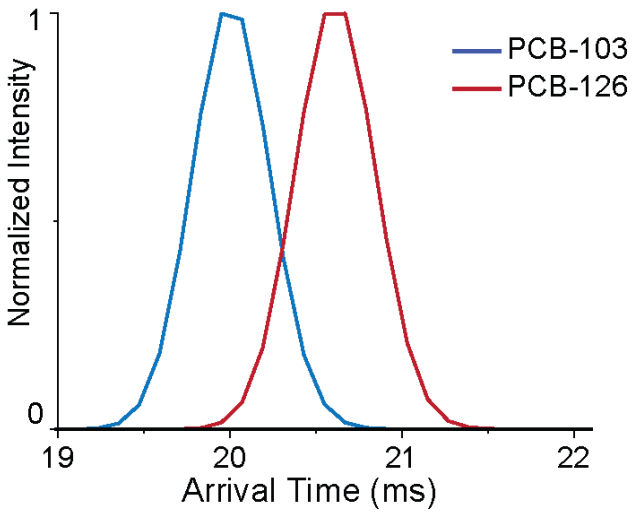
α-D-Glucose-1-phosphate (G1P)



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

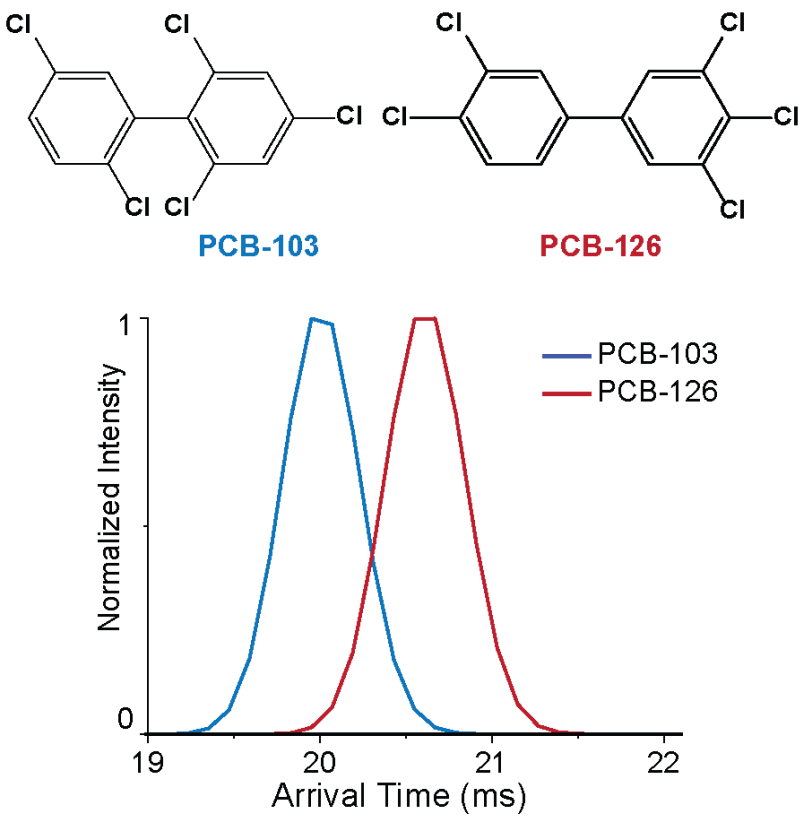
Isomeric xenobiotic separations

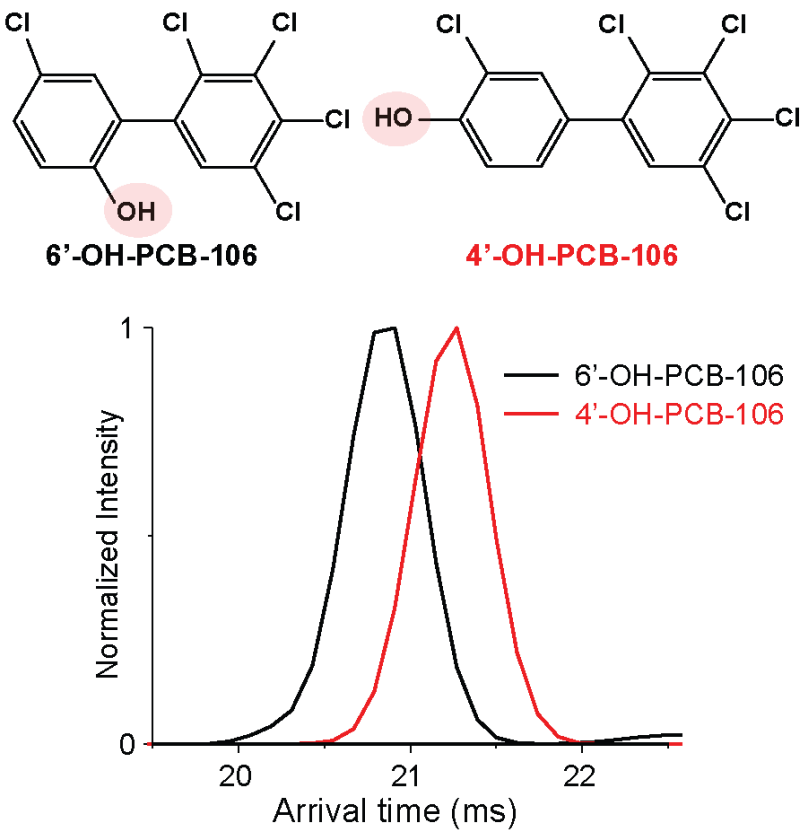




Collaboration with Keri Hornbuckle's Group

Isomeric xenobiotic separations

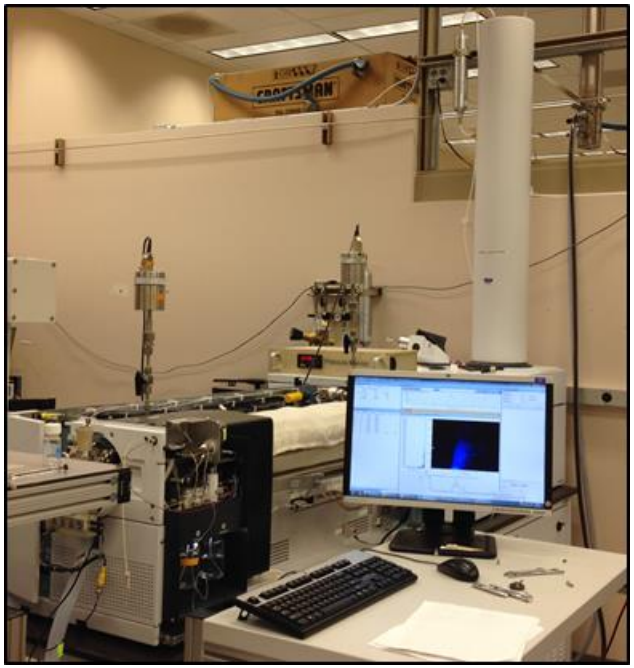




Collaboration with Keri Hornbuckle's Group

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

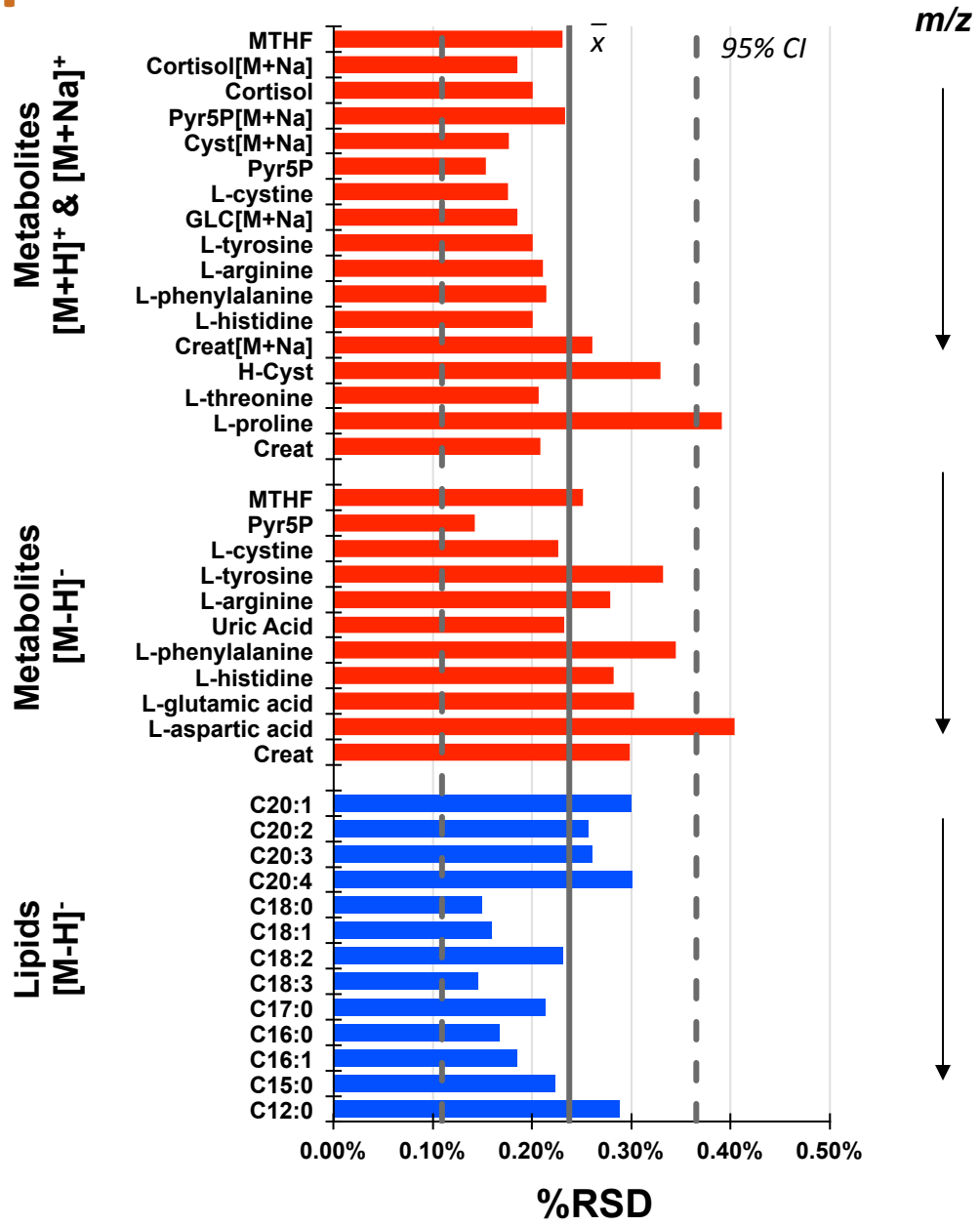


Pacific Northwest National Laboratory, Richland
Agilent Technologies, Inc., Santa Clara
Vanderbilt University, Nashville
BOKU – Univ. of Natural Resources and Life Sciences, Vienna

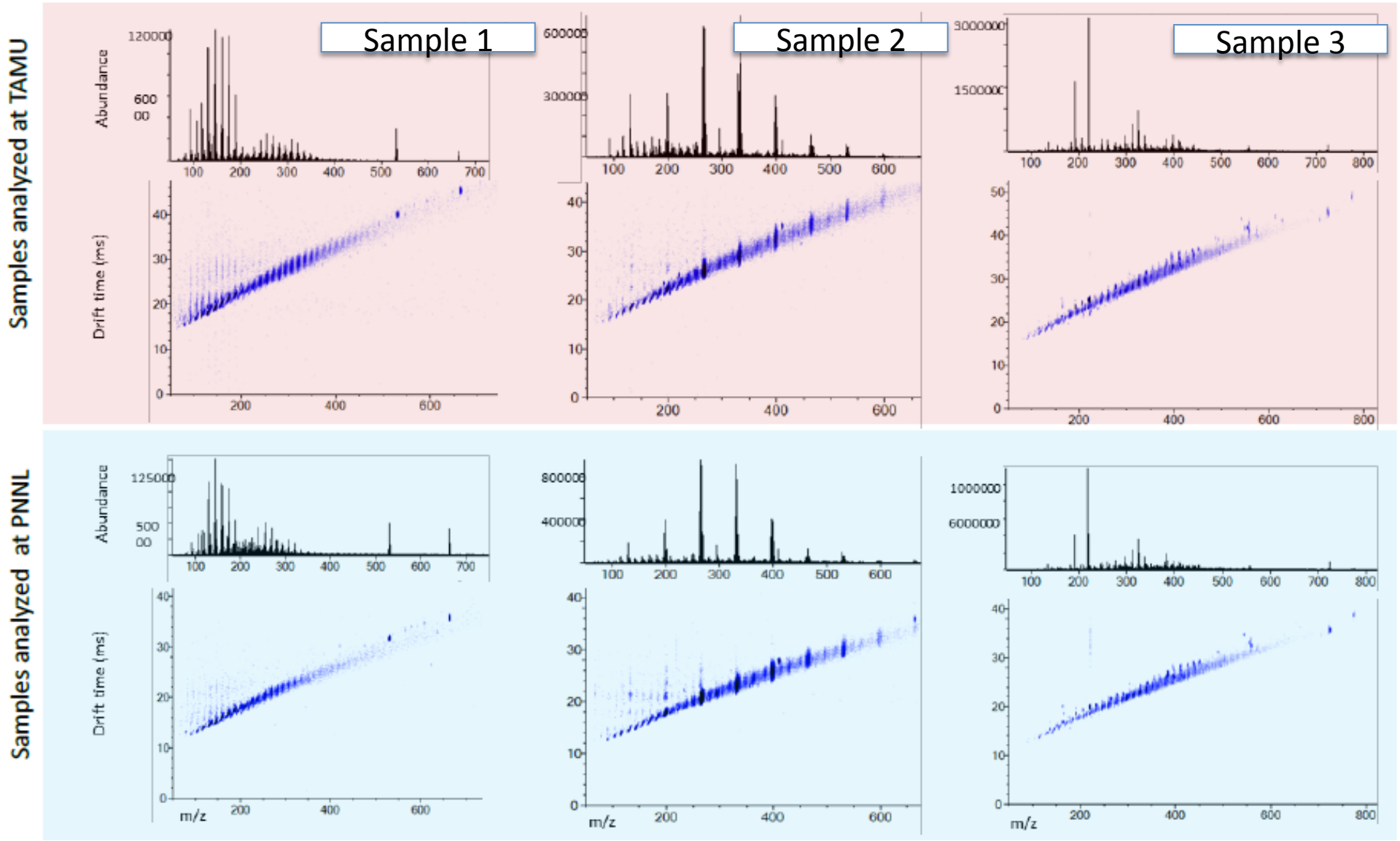
S. M. Stow, et al., *Anal. Chem.* **2017**, 89, 9048-9055.

Interlab precision comparison

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

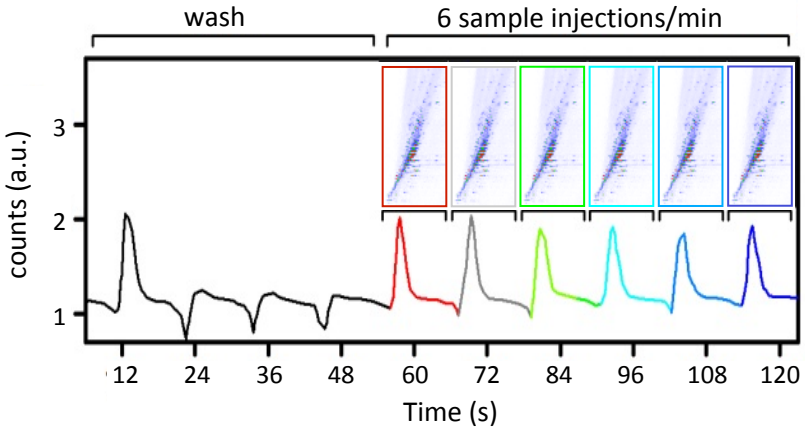


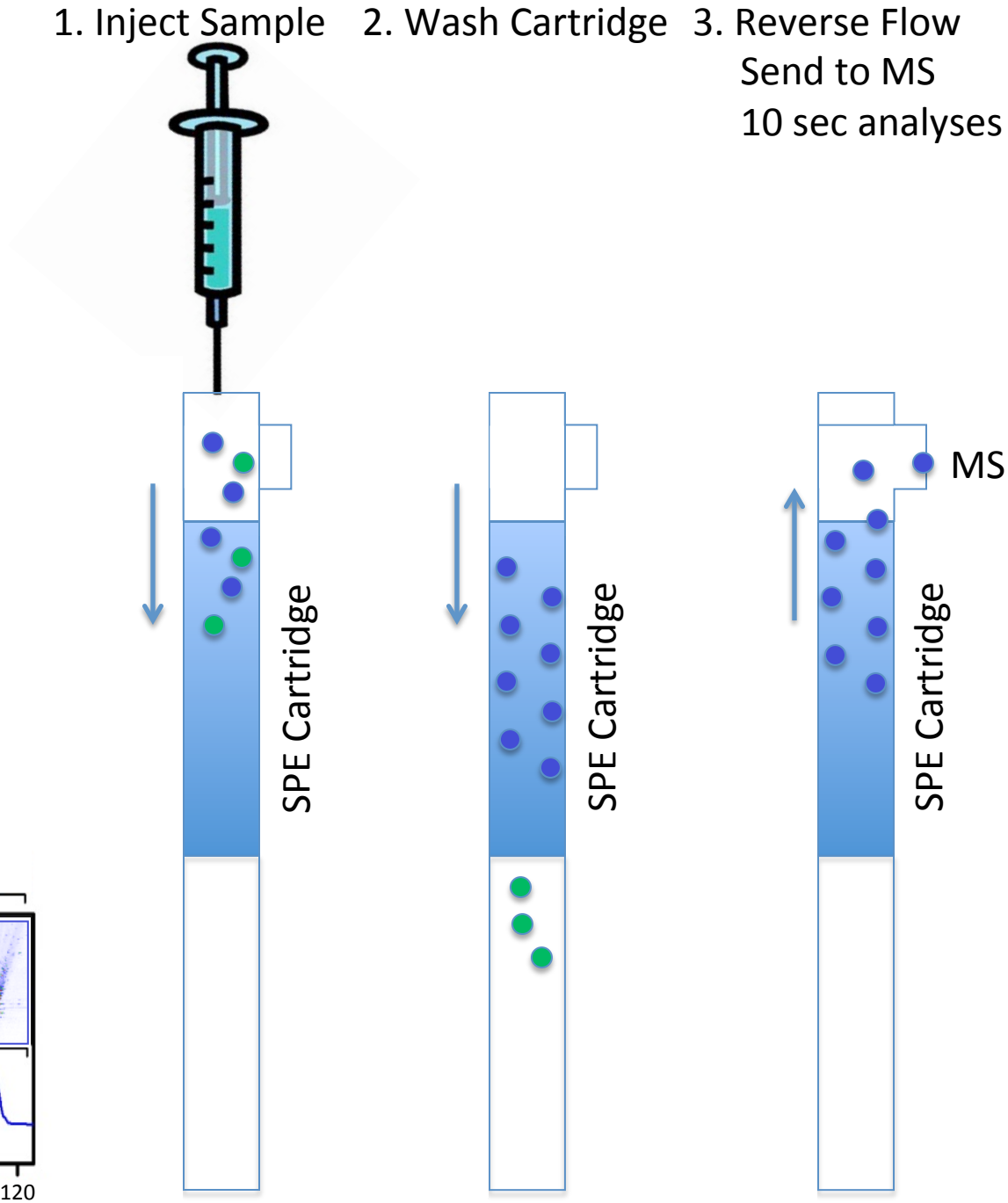
Interlab mixture reproducibility



Collaboration with Ivan Rusyn's Group

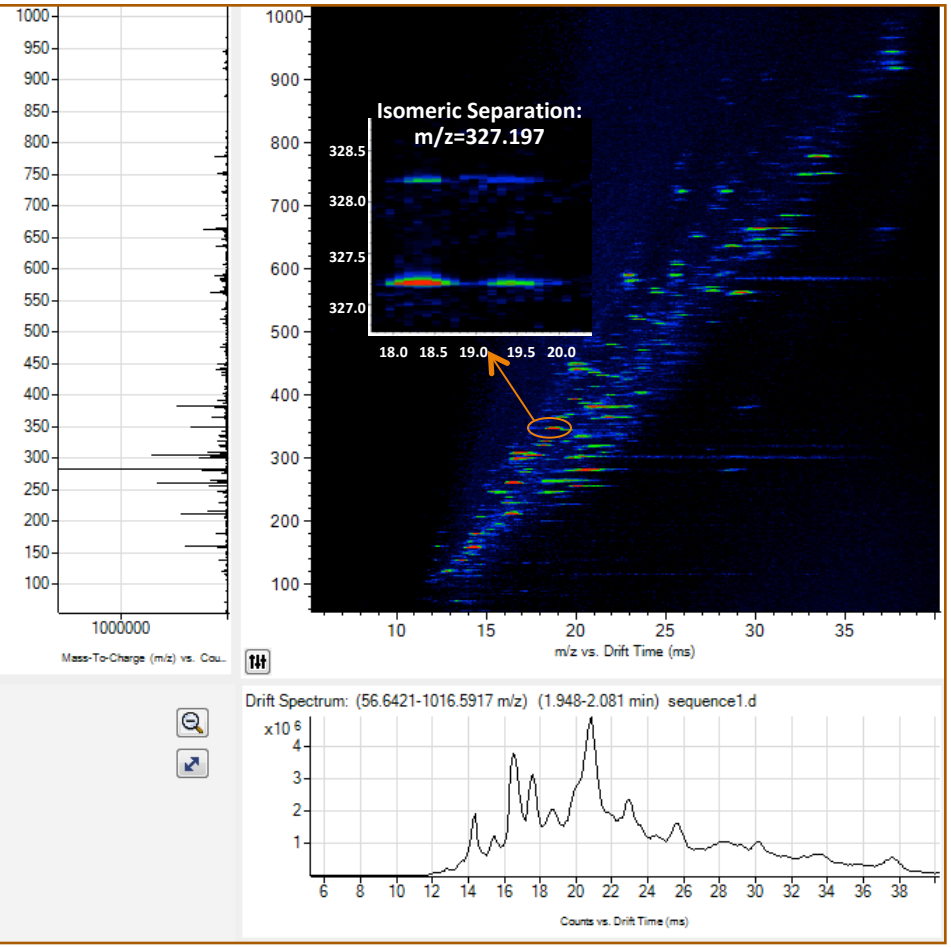
Automated SPE system





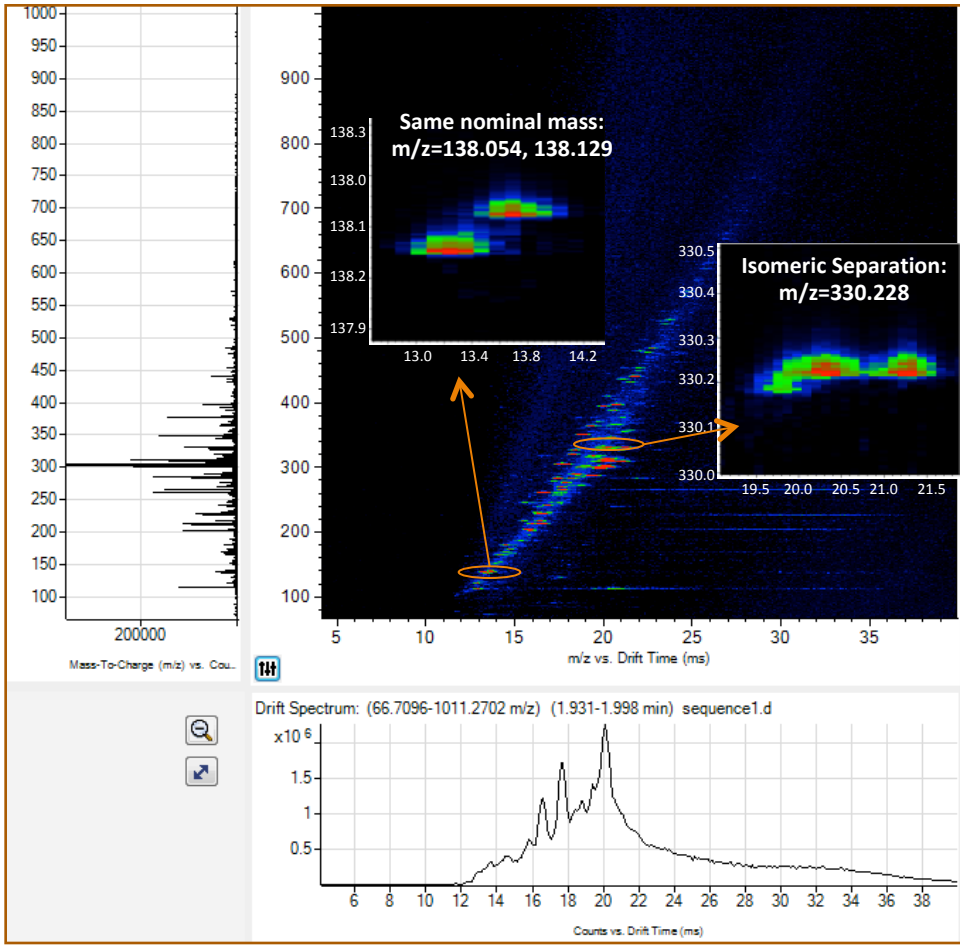
SPE-IMS-MS analyses of biological samples

Polar molecules extracted from mouse plasma



~1400 features with S/N > 5

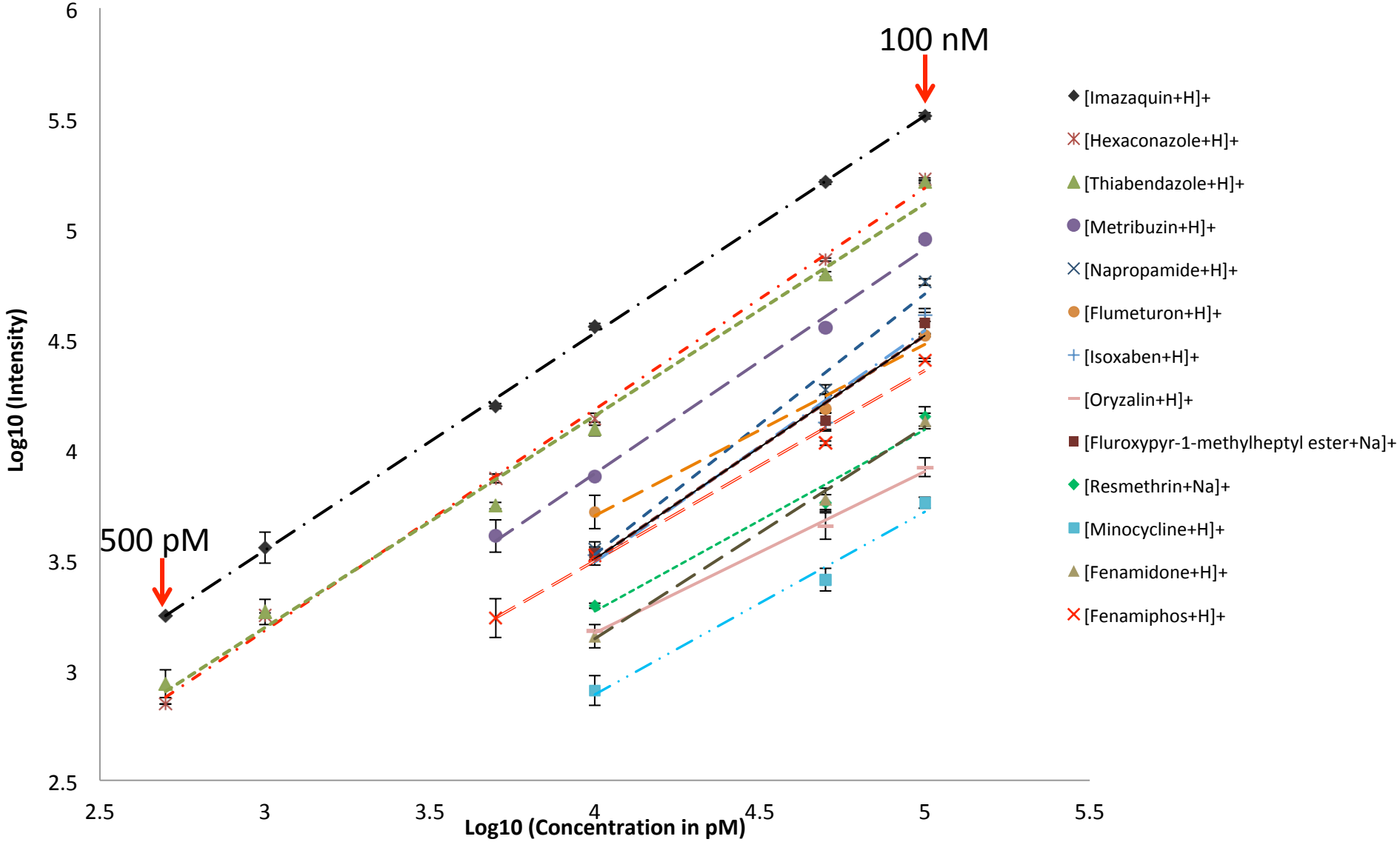
Polar molecules extracted from human urine



~1000 features with S/N > 5

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

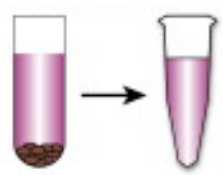
Calibration curve for xenobiotics in plasma



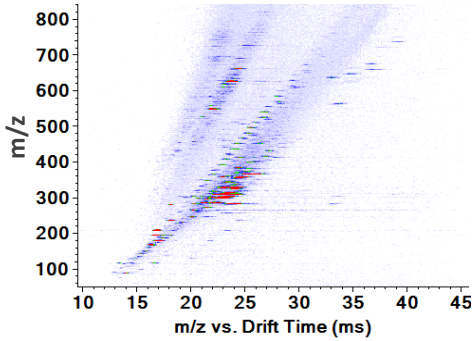
X. Zhang, et al., *Clin. Mass Spectrom.* **2016**, 2, 1-10.

Small molecule pipeline

Extraction



Instrumental Analysis



Data Processing & Analysis

ID	Mass	Intensity
----	----	----
----	----	----
----	----	----
----	----	----
----	----	----

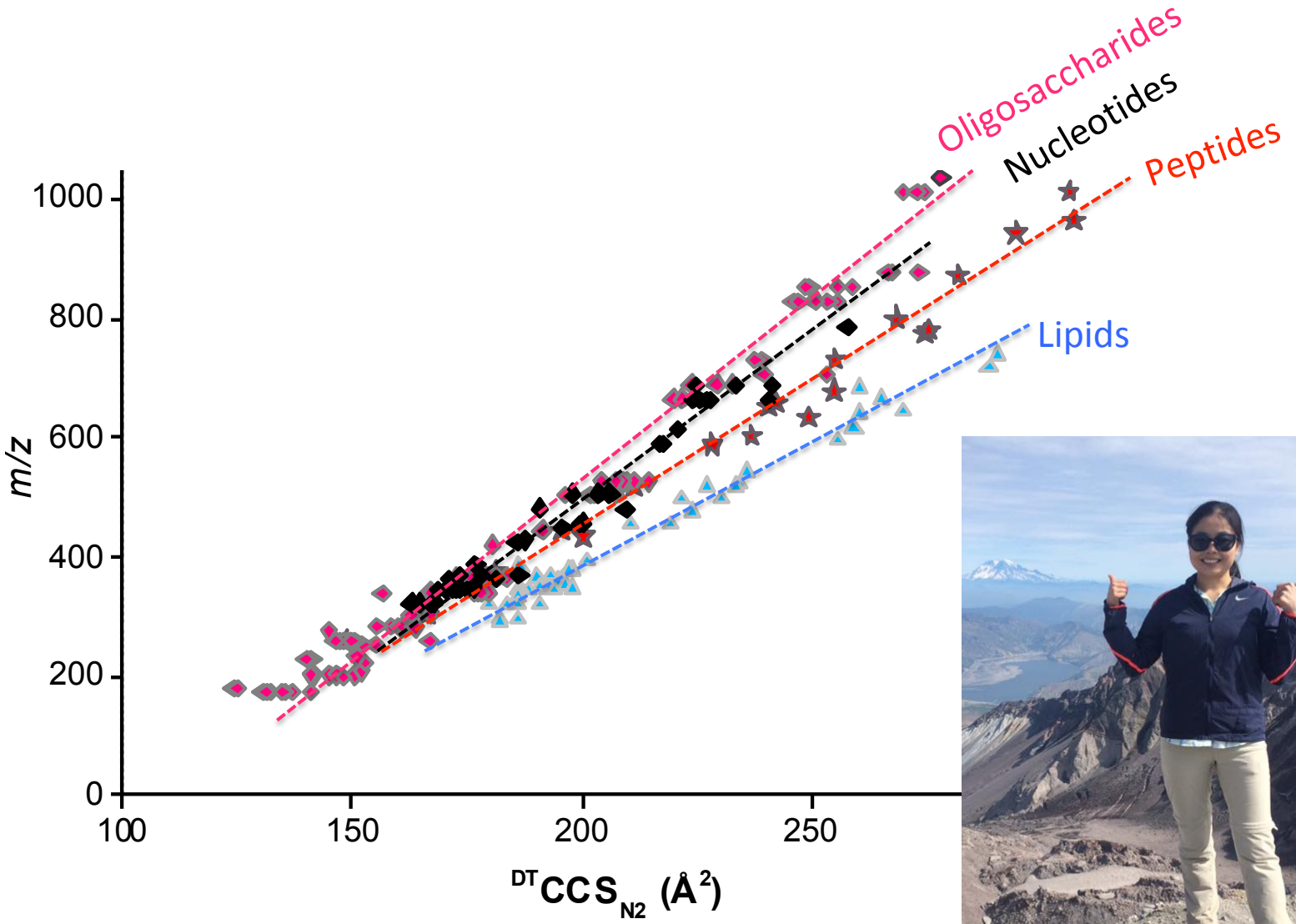
Methanol/Chloroform Extraction
(100 samples/day)

SPE-IMS-MS

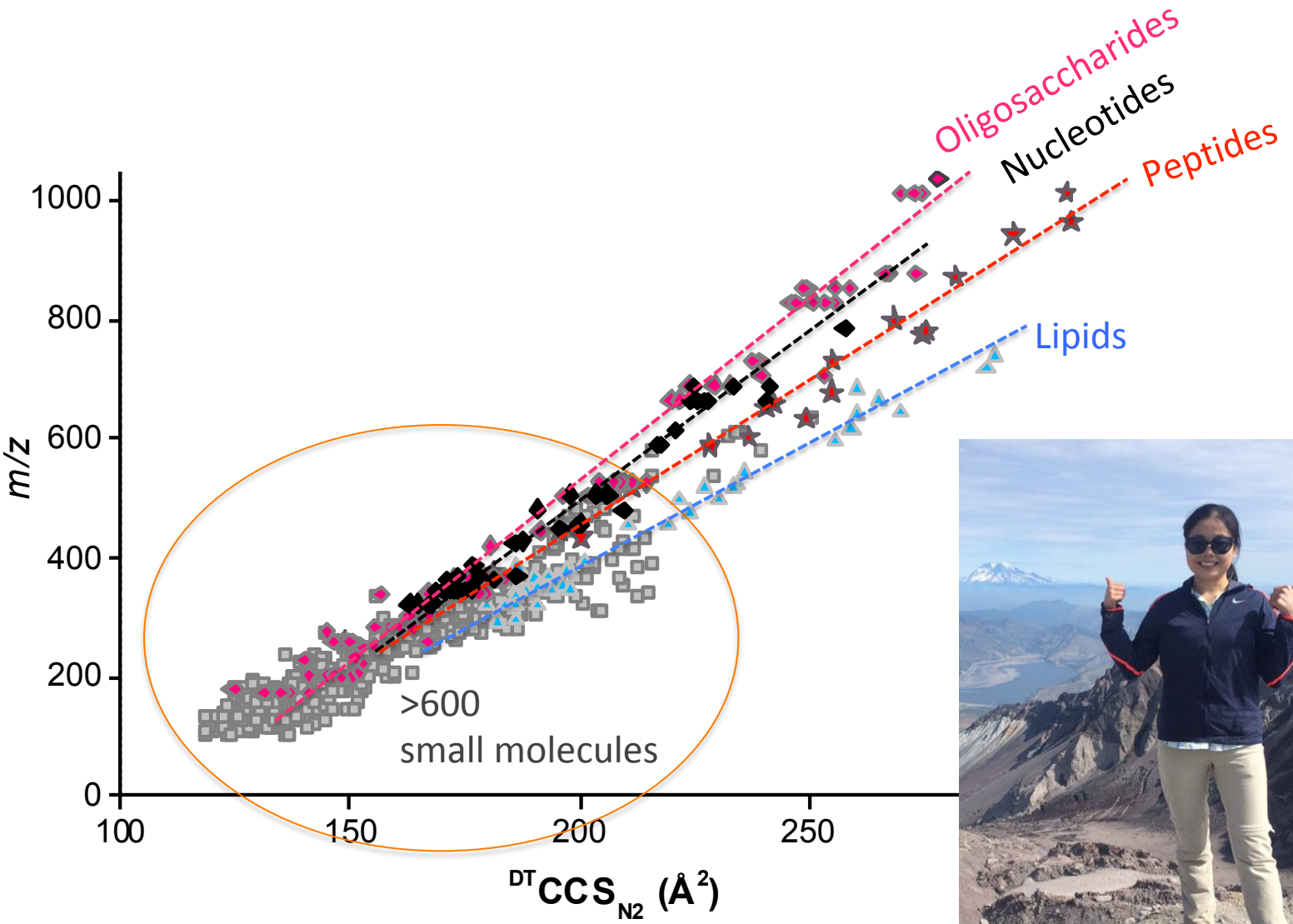
(8200 samples/day)

Database Matching & False Discovery Assessment
(? days)

Collision cross section database

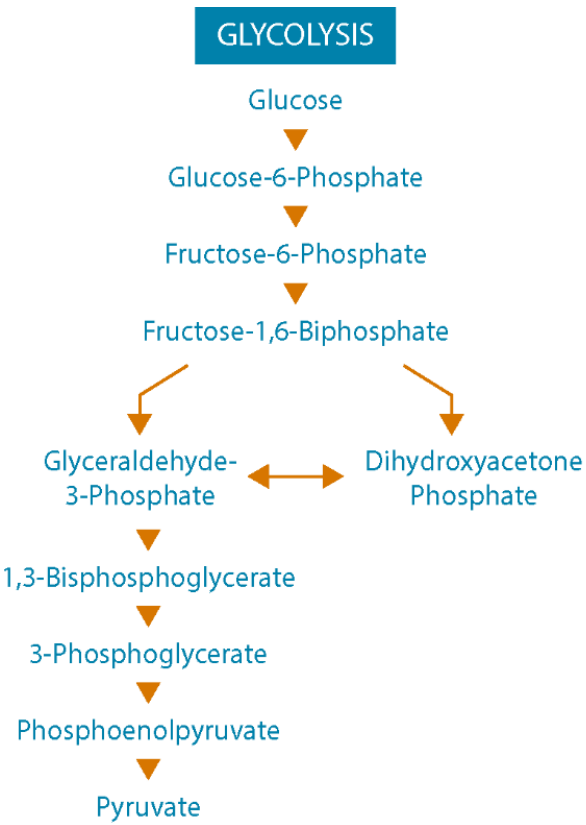


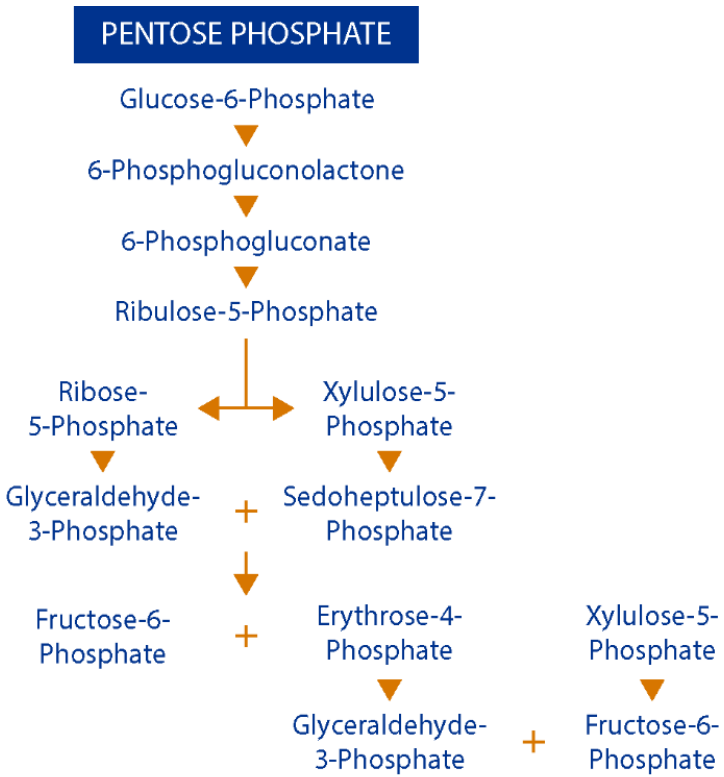
Collision cross section database

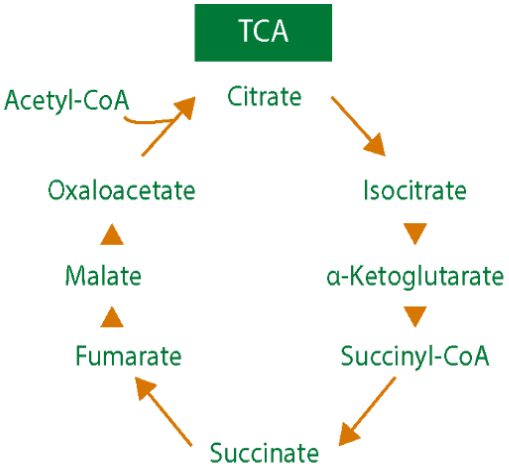


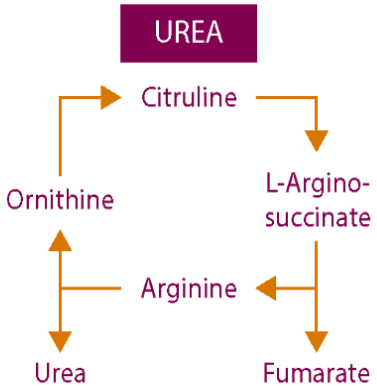
X. Zheng, et al., *Chem. Sci.* **2017**, ASAP.

Metabolic pathways



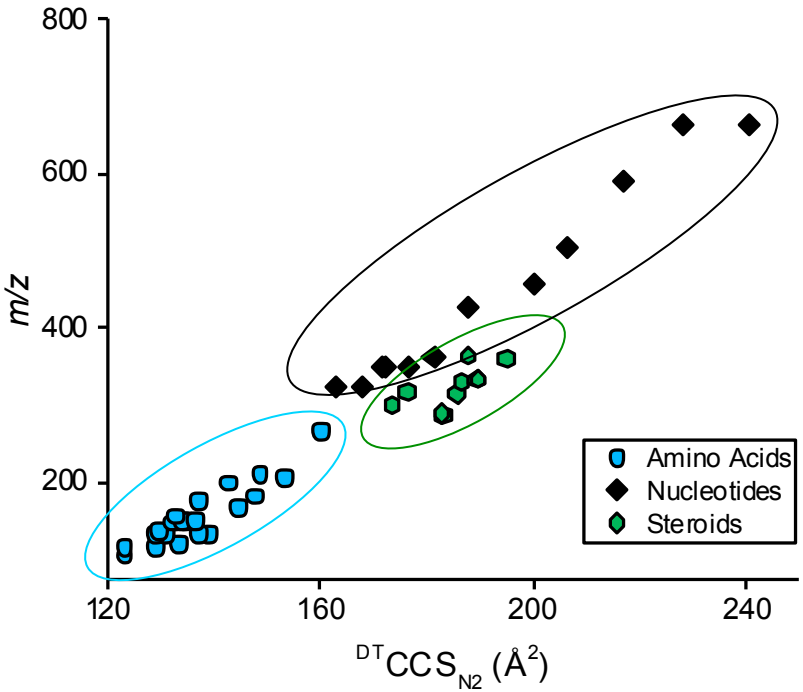






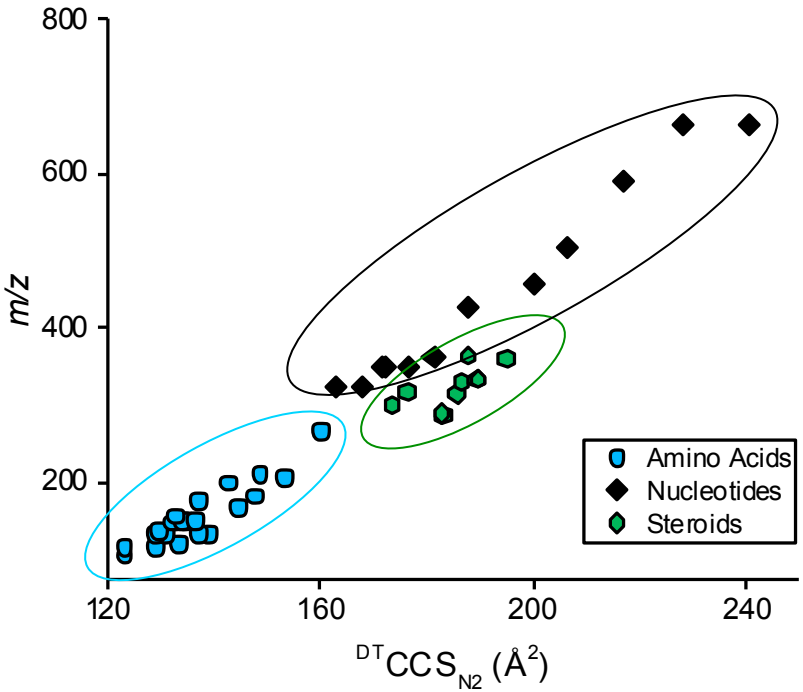
Primary metabolite trend lines

Protonated form

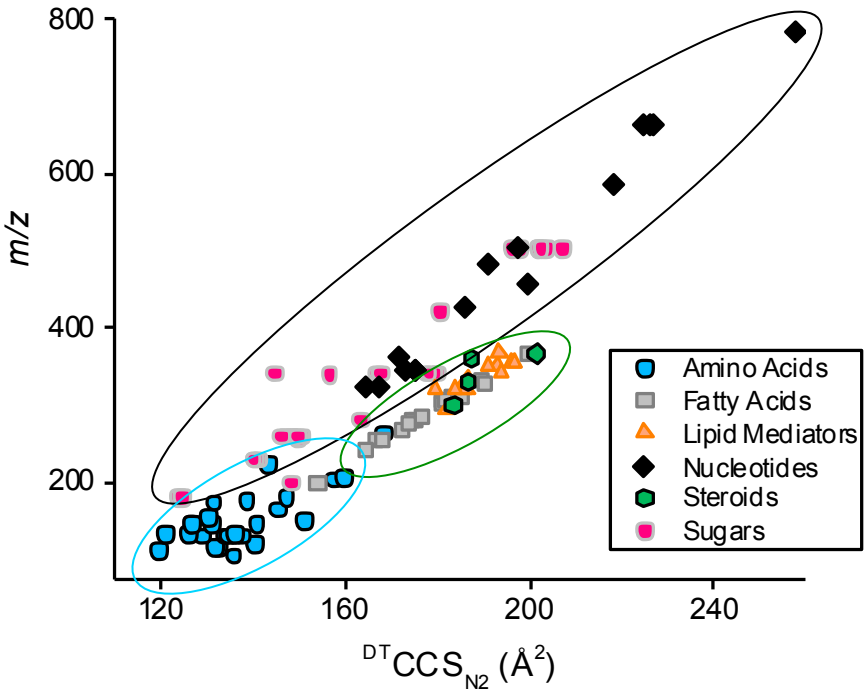


Primary metabolite trend lines

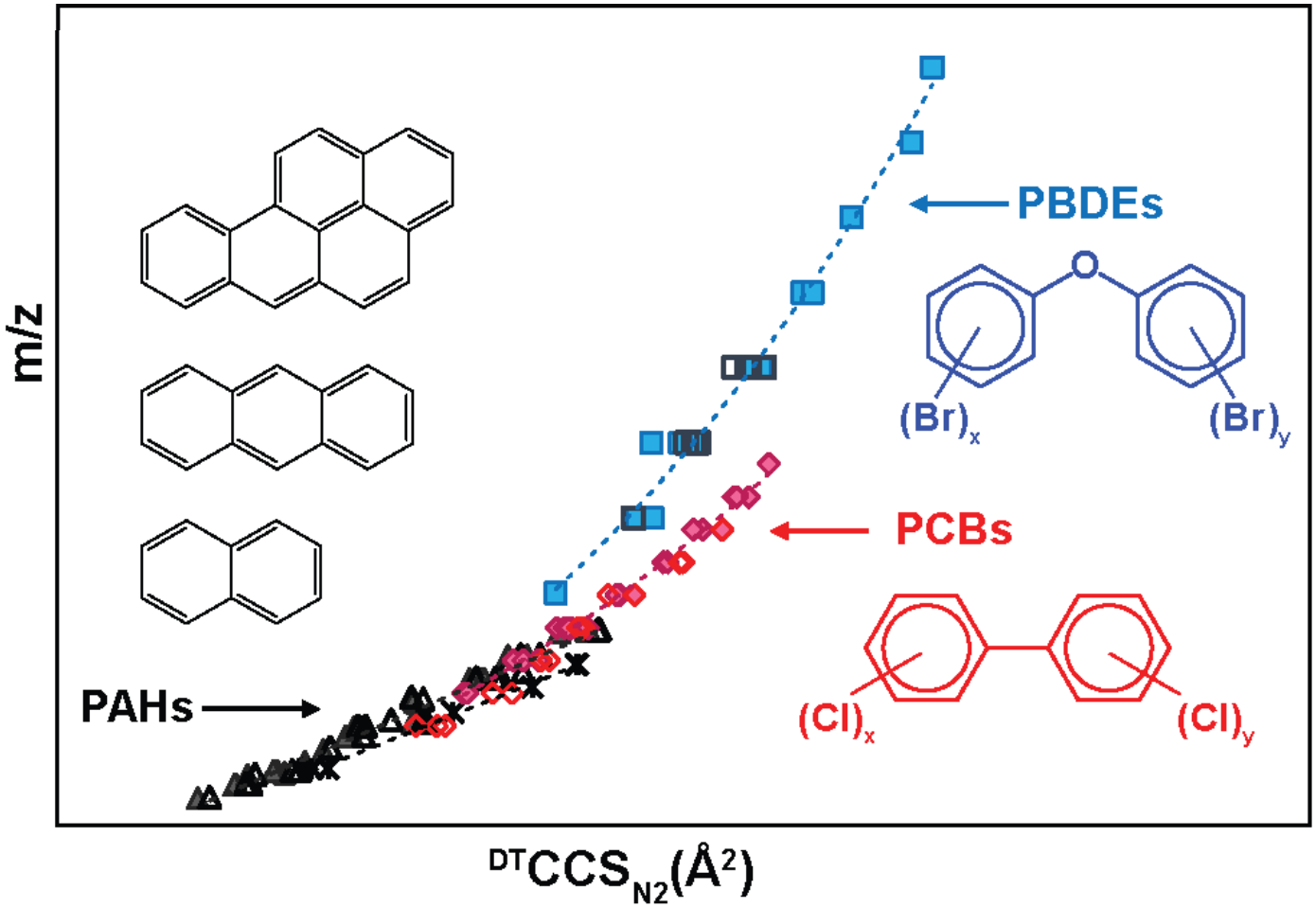
Protonated form



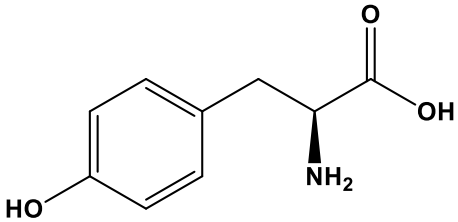
Deprotonated form



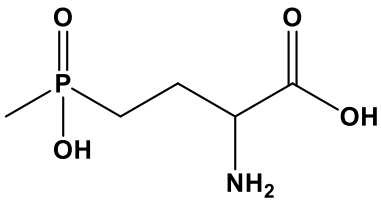
Xenobiotic trend lines



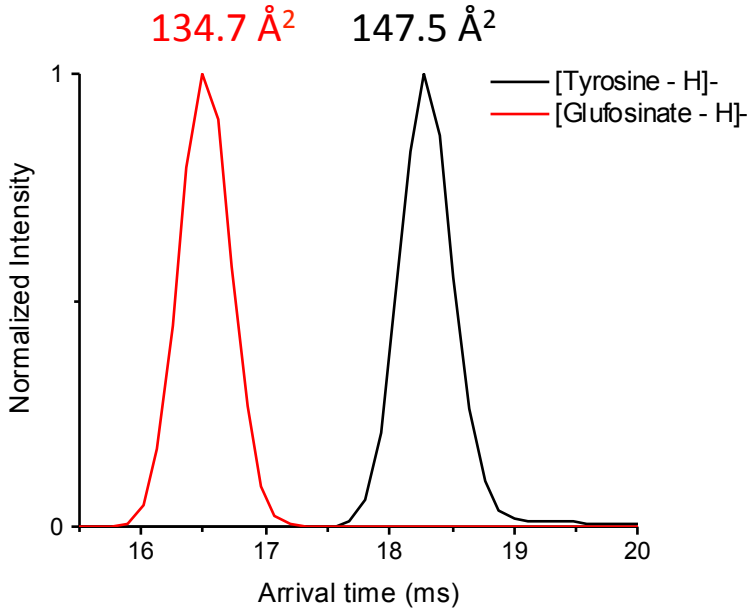
Primary metabolite versus xenobiotic



L-Tyrosine (amino acid)
Exact mass: 181.0738896



Glufosinate (herbicide)
Exact mass: 181.0503922



Deprotonated form

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

Pacific Northwest NATIONAL LABORATORY
Proudly Operated by Battelle *Since 1965*

U.S. DEPARTMENT OF ENERGY

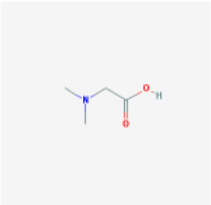
PNNL Home | About | Research | Publications | Jobs | Newsroom | Contacts Search PNNL

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

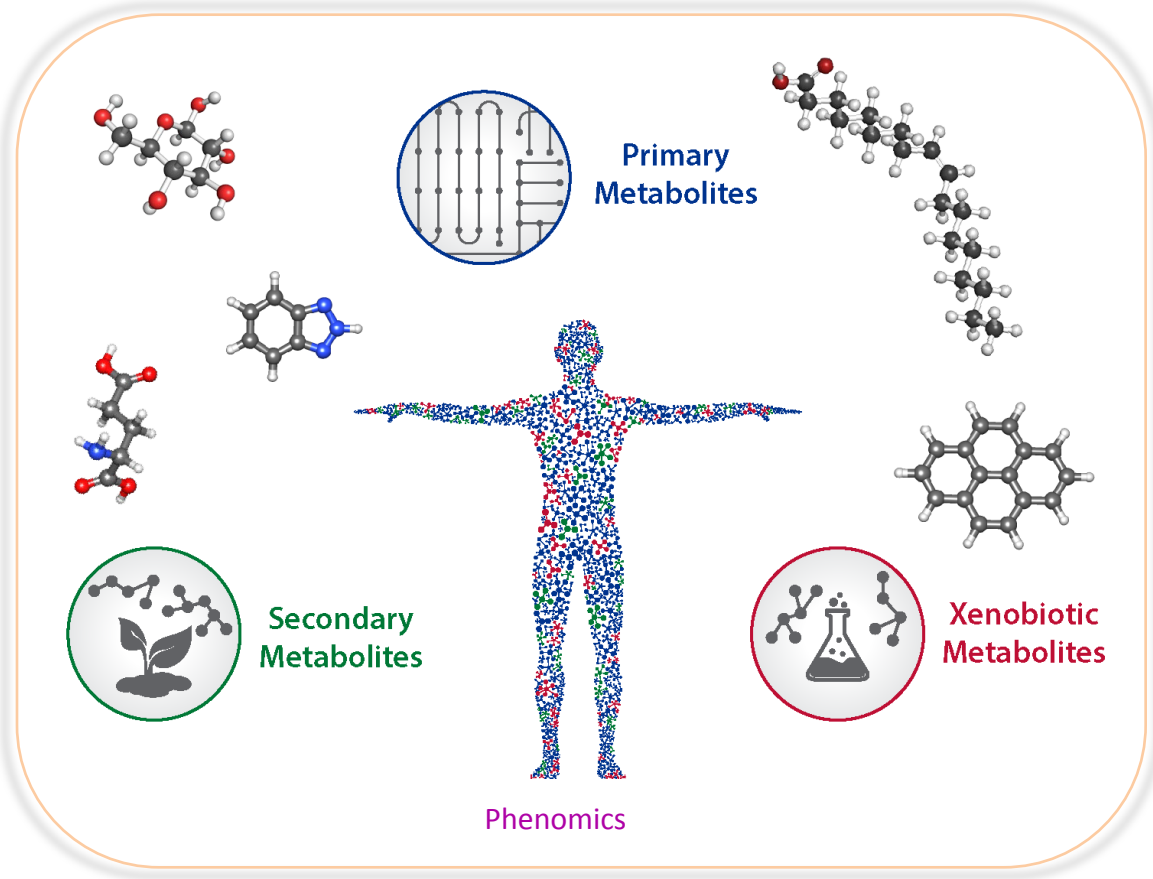
Primary Metabolite ✕ | Subclass | Search:

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)



Acknowledgements

- Agilent Technologies
- NIEHS R01ES022190
- NIEHS Superfund Research Program P42 ES027704
- NIH General Medical Sciences P41 GM103493-11
- PNNL Laboratory Directed Research and Development Program
- Environmental Molecular Sciences Laboratory

Noor Aly