Using Multidimensional Separations to Identify Chemicals in Non-Targeted Analyses

Baker Laboratory
Est. 2018
Mass-to-Charge (m/z)
Collision Cross Section (Å²)

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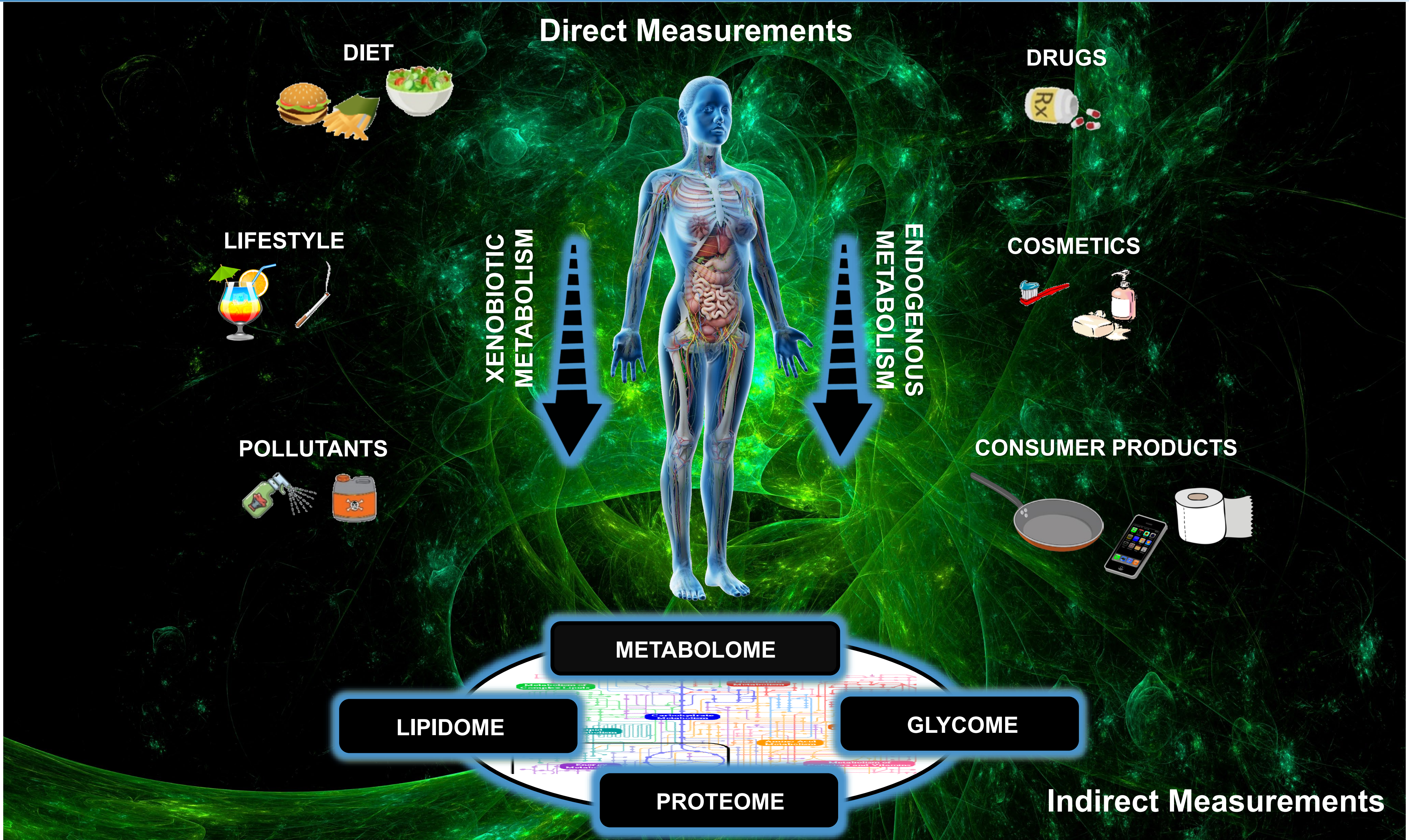
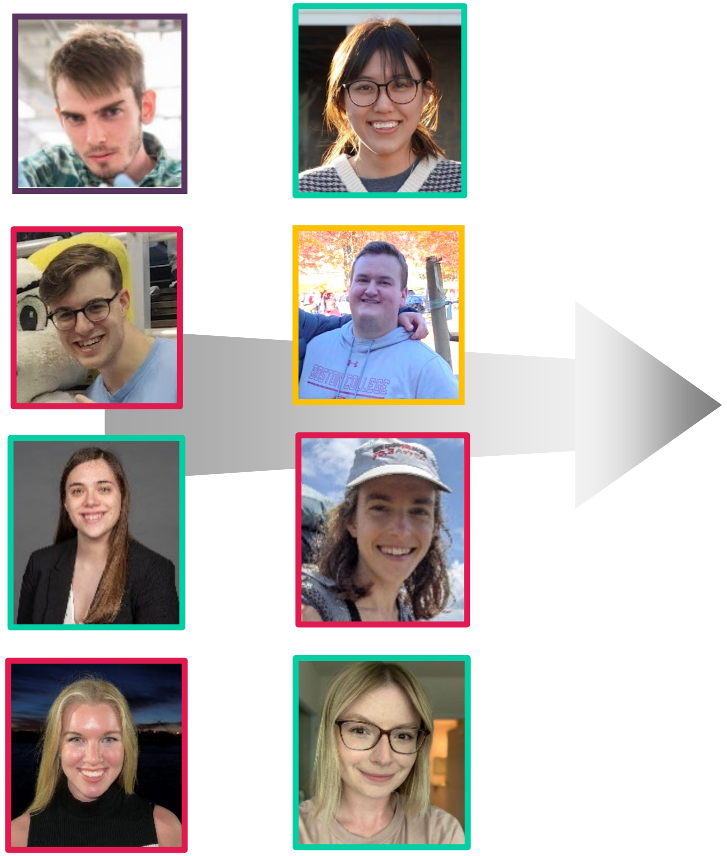
TEXAS A&M UNIVERSITY
SUPERFUND
RESEARCH CENTER

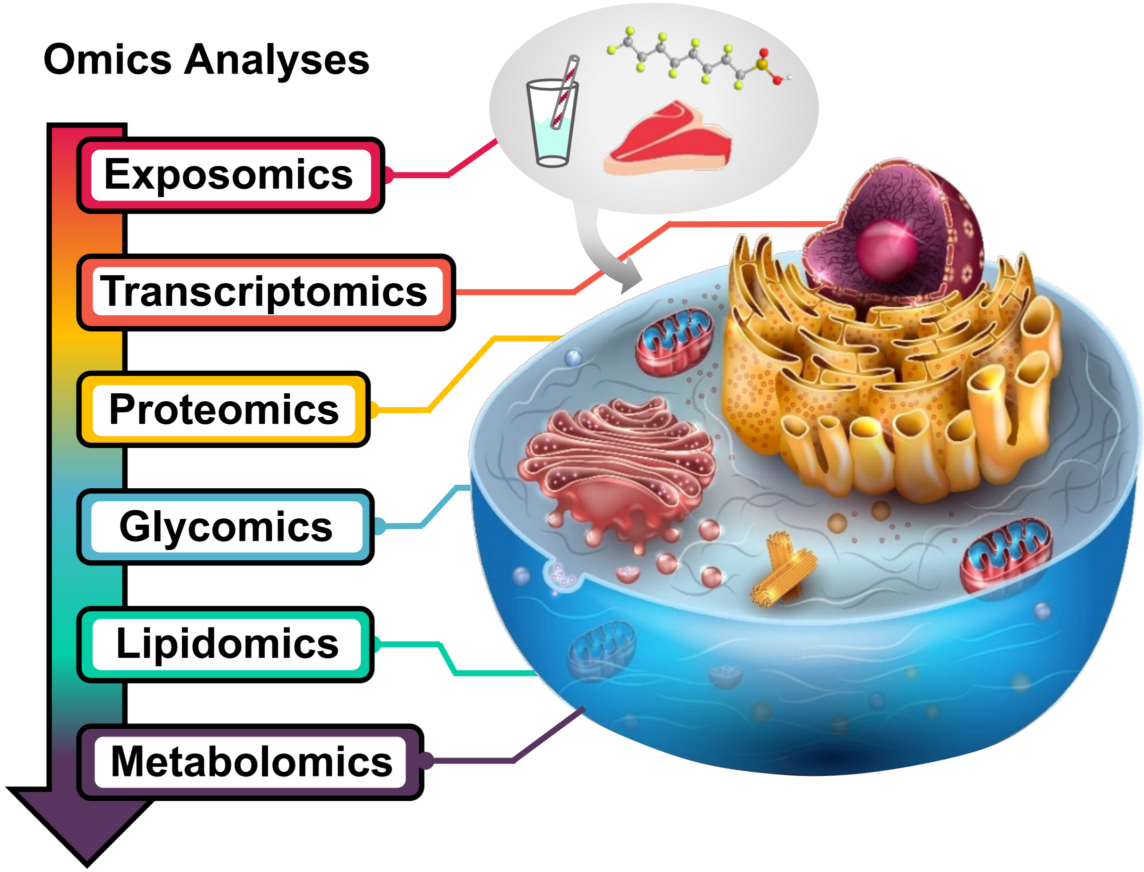
Chemical Exposure and Human Health



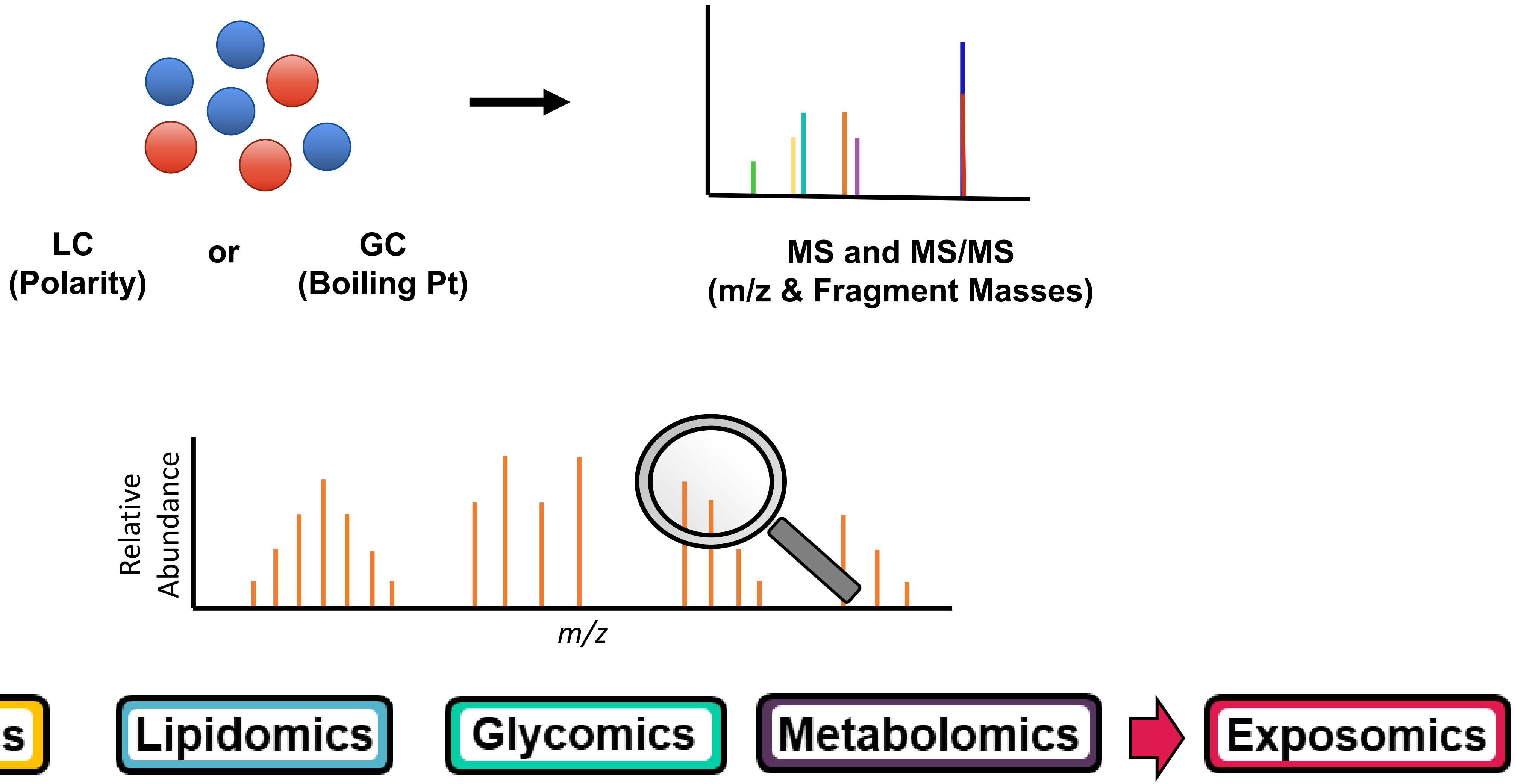
Multi-Omics in the Baker Lab

- Multi-Omic profiling enables a greater understanding of system perturbations



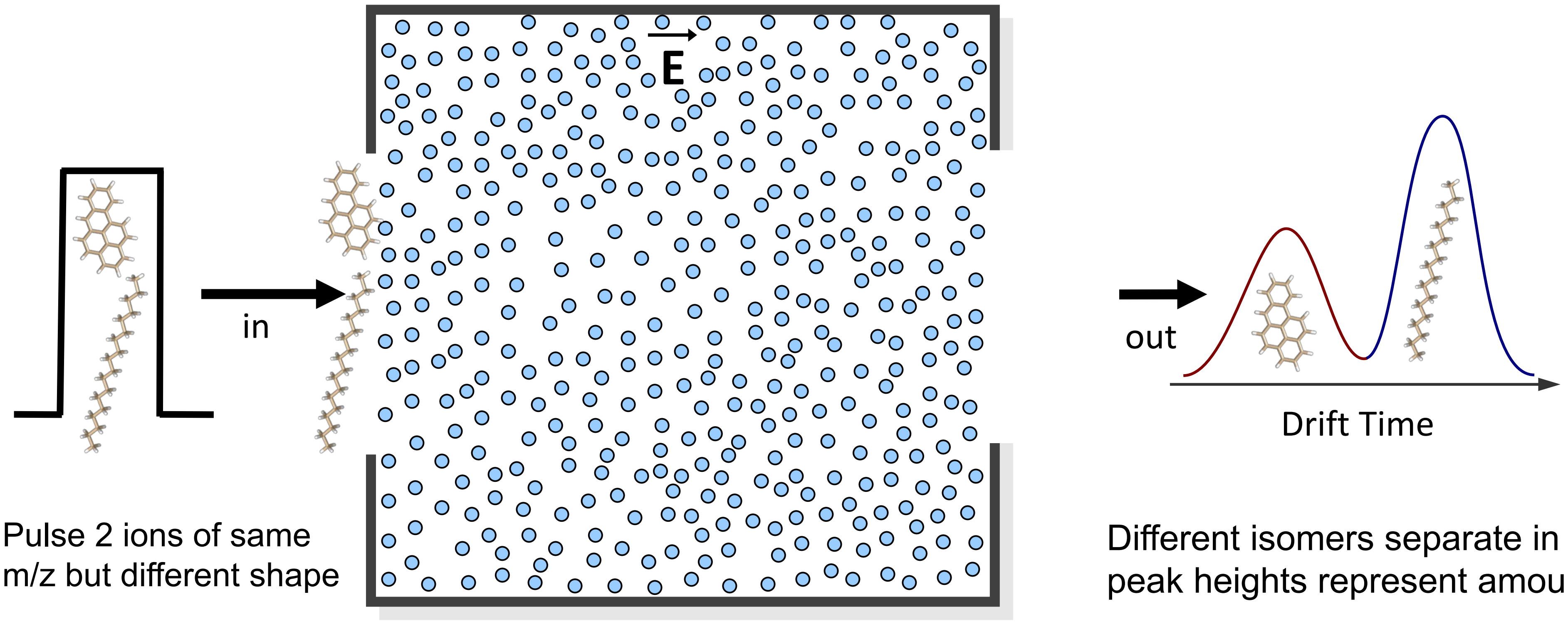


Chromatography and Mass Spectrometry Workflow



Ion Mobility Spectrometry: A Unique Approach to Chemical Analyses

- IMS is a **very fast** separation technique which provides **structural information**



Pulse 2 ions of same m/z but different shape

Drift Cell

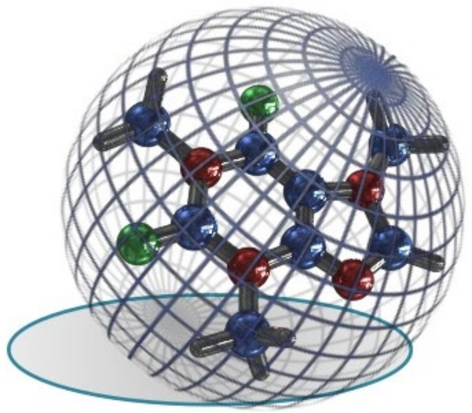
Different isomers separate in time & peak heights represent amounts

Velocity is constant

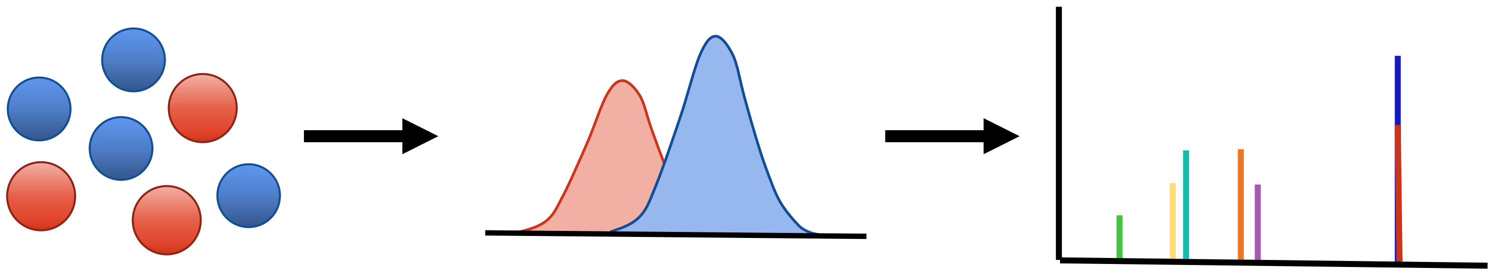
How Can IMS Help Molecular Analyses?

1. IMS millisecond time scale is easily coupled between front-end separations & mass spectrometry

2. Ion-neutral collision cross sections (CCSs) provide an added dimension



3. IMS has many benefits for omic analyses

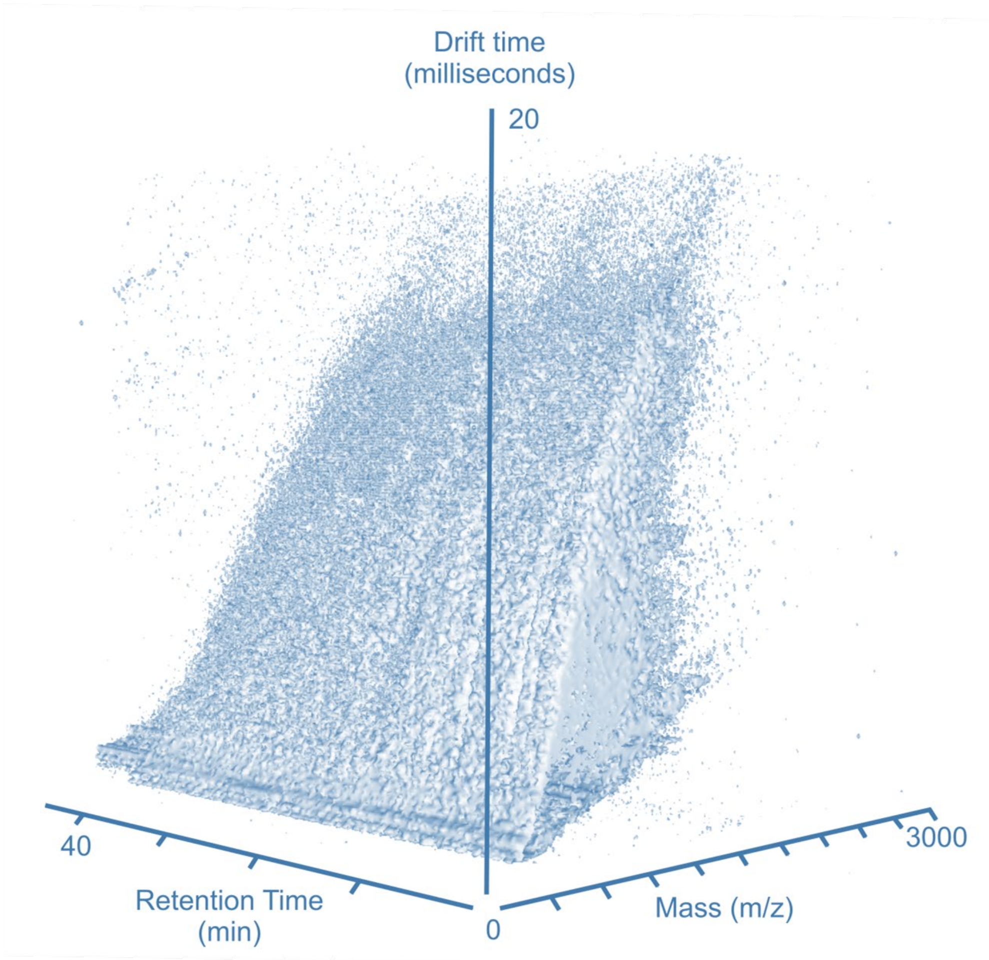


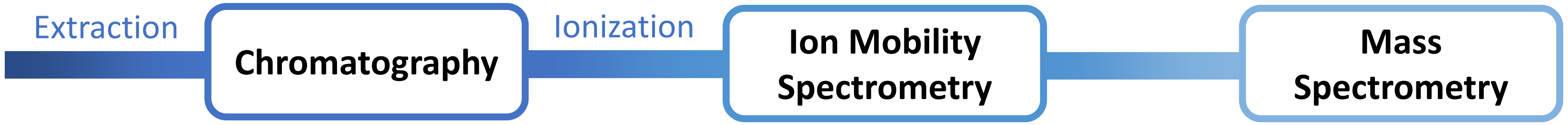
LC
(Polarity)

Ion Mobility
(Size)

MS and MS/MS
(m/z & Fragment Masses)

High Dimensional Molecular Characterization = Big Data

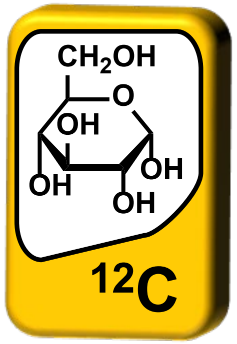


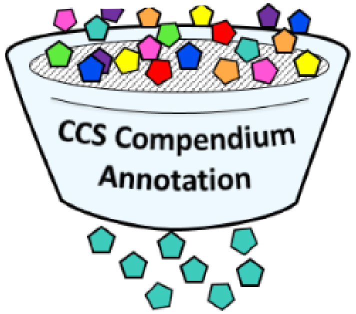


Fenn, L.S.; McLean, J.A. *Anal. Bioanal. Chem.* 2008, 391, 905.

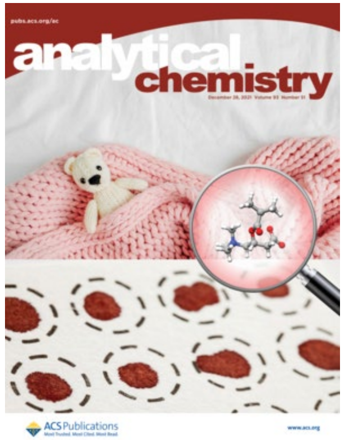
Molecular Databases and Libraries of CCS from IMS Studies

>3800 Metabolites
McLean Lab (VU)





Picache, J.A. et al. *Chem. Sci.*, 2019, *10*, 983.

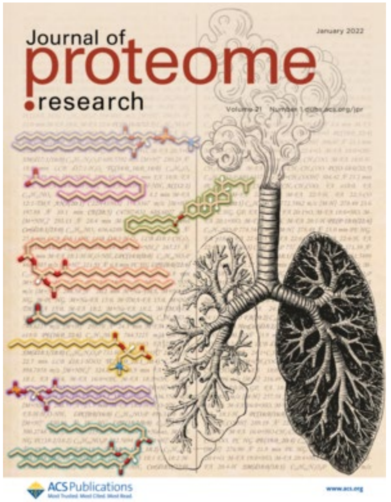


>600 Metabolites
Baker Lab (PNNL)

Zheng, X. et al. *Chem. Sci. 2017, 8, 7724.*

41 Acylcarnitines (NBS)
Baker Lab (UNC)

Dodds, J.N. et al. *Anal. Chem.* 2021, *93*, 17094.

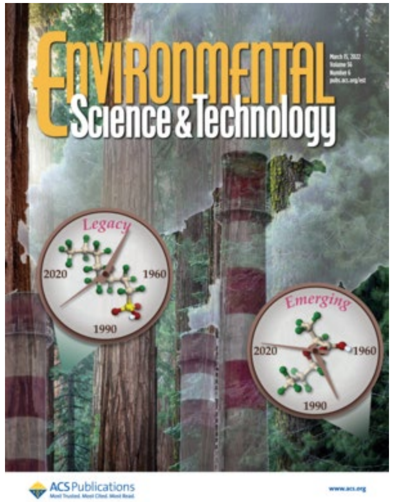


>1000 Lipids
Baker Lab (UNC)

Kirkwood, K.I. et. al.
J. Proteome Res. 2022, *21*, 232.

>200 Bile Acids
Baker Lab (UNC)

Stewart, A. et al.
Anal. Chem. 2023, ASAP.



>100 PFAS
Baker Lab (UNC)

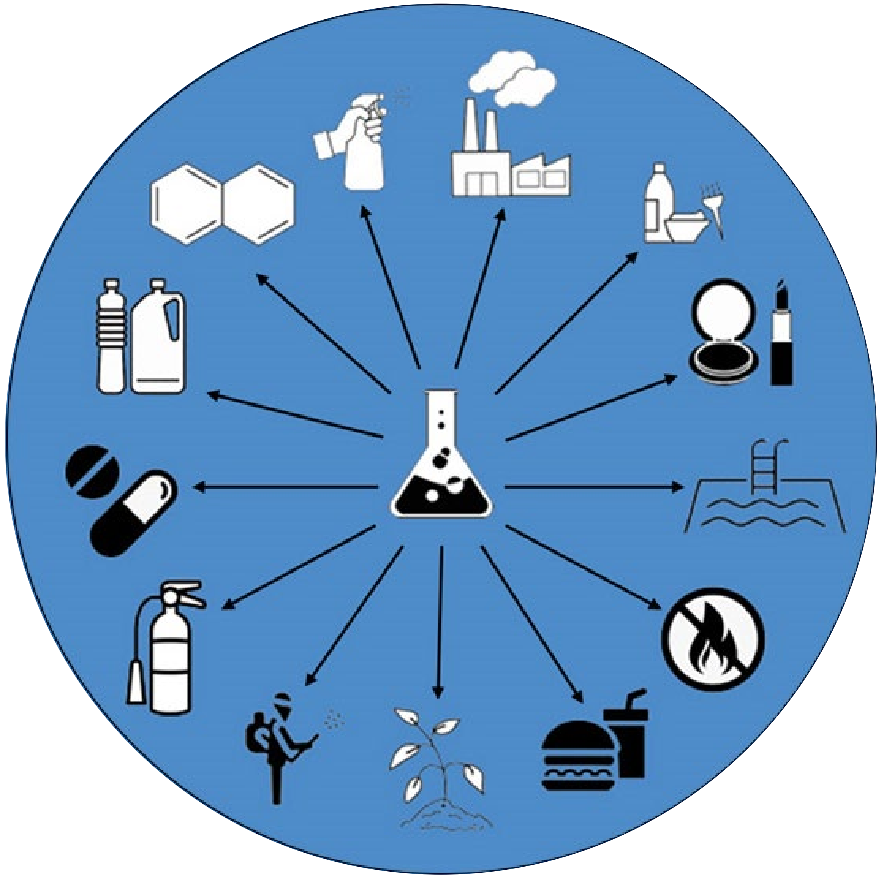
Kirkwood, K.I. et. al. *Environ. Sci. Technol.* 2022, *56*, 3441-3451.

4685 ToxCast
Rusyn Lab (TAMU)

Underway

Environmental Exposomic Molecular Database

ToxCast Chemical Library = 4,685
Chemical Classes = 13



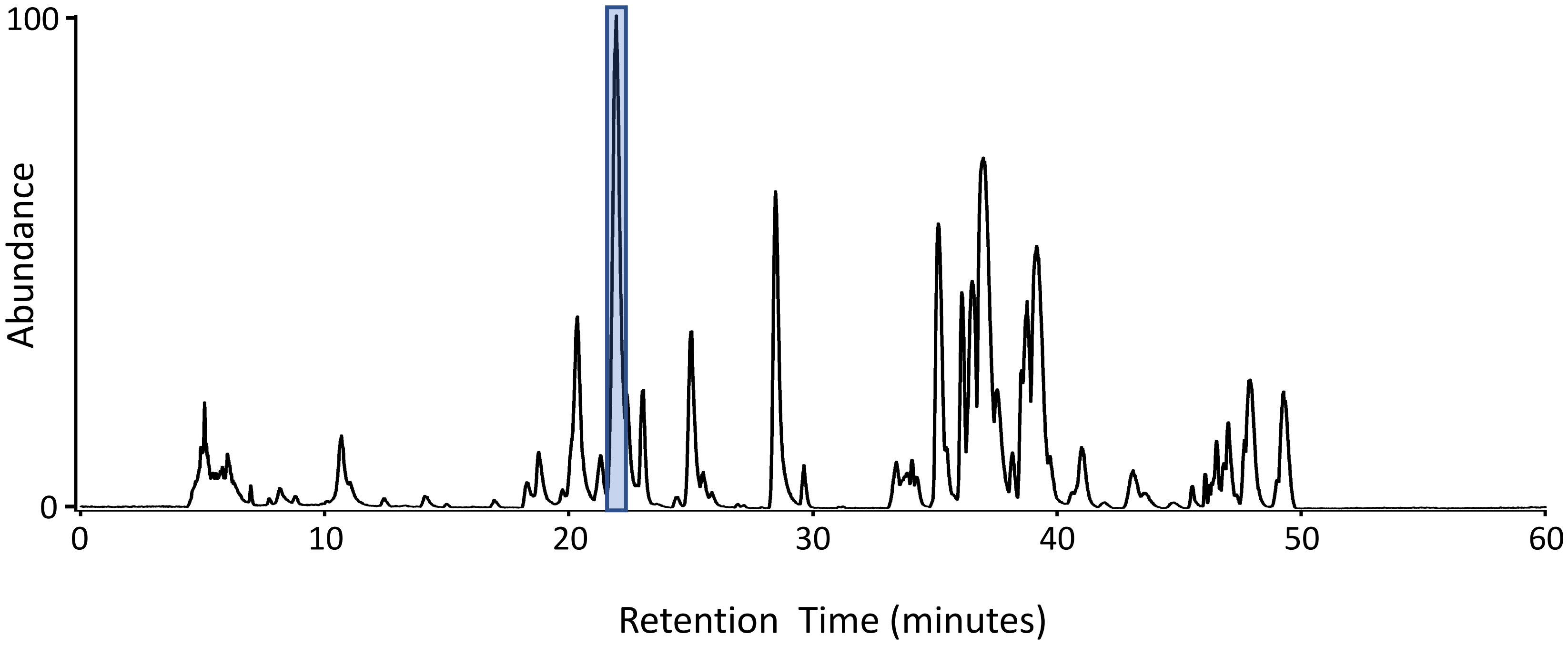
Chemicals Industrial	Color Dyes
Cosmetic Ingredients	Disinfection By-Products
Flame Retardants	Food Additives
Natural Toxins	Pesticides
PFASs	Pharmaceuticals
Plastics	PAHs
Surfactants	

- Reproducibility ±1% for Collision Cross Sections

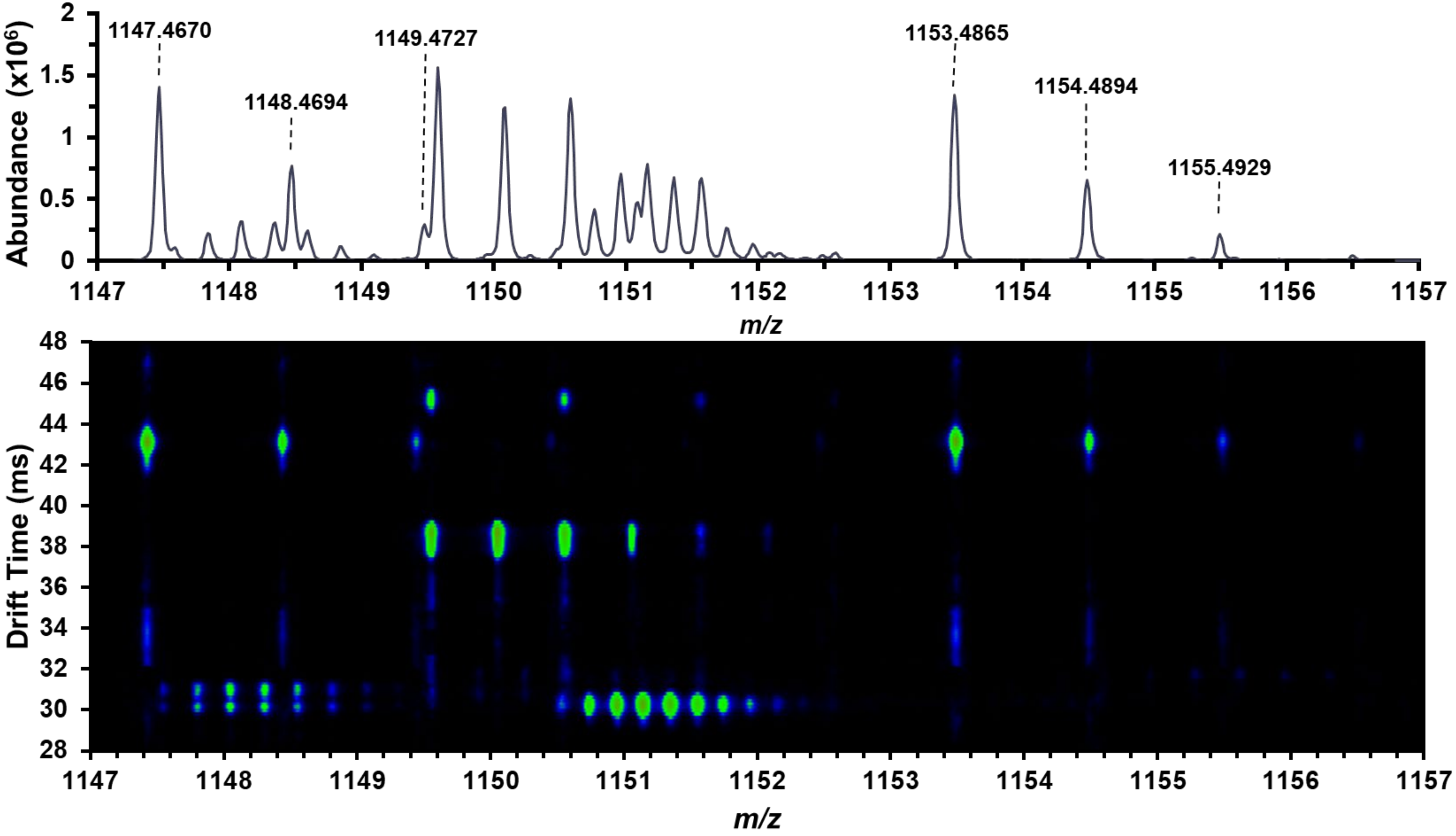
Ivan Rusyn
Texas A&M

Interference Removal and Increased Confidence

- LC-IMS-MS Data

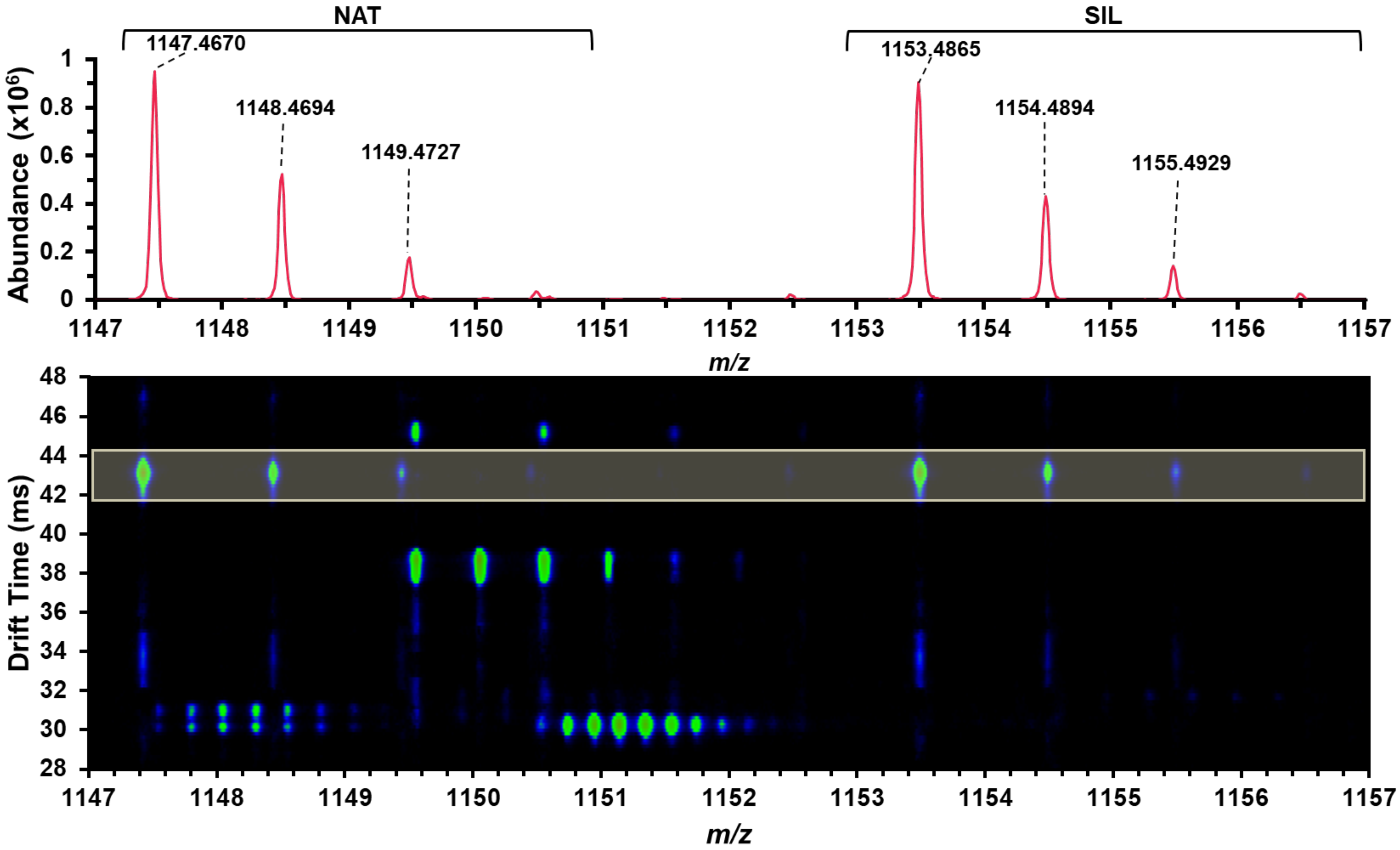


Interference Removal and Increased Confidence



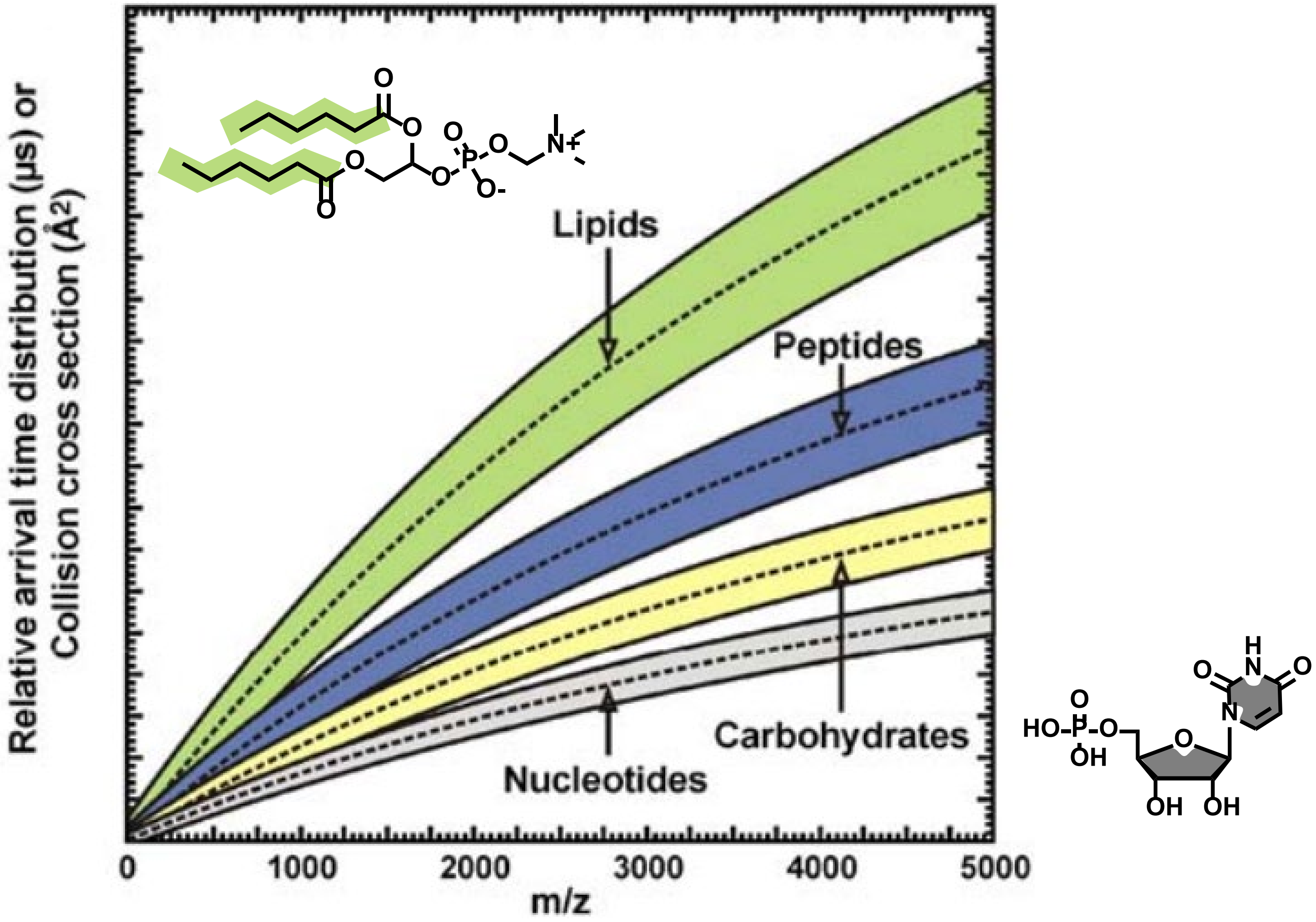
Butler, K.E., et al. *Anal. Bioanal. Chem.* 2021, 414, 623.

Interference Removal and Increased Confidence



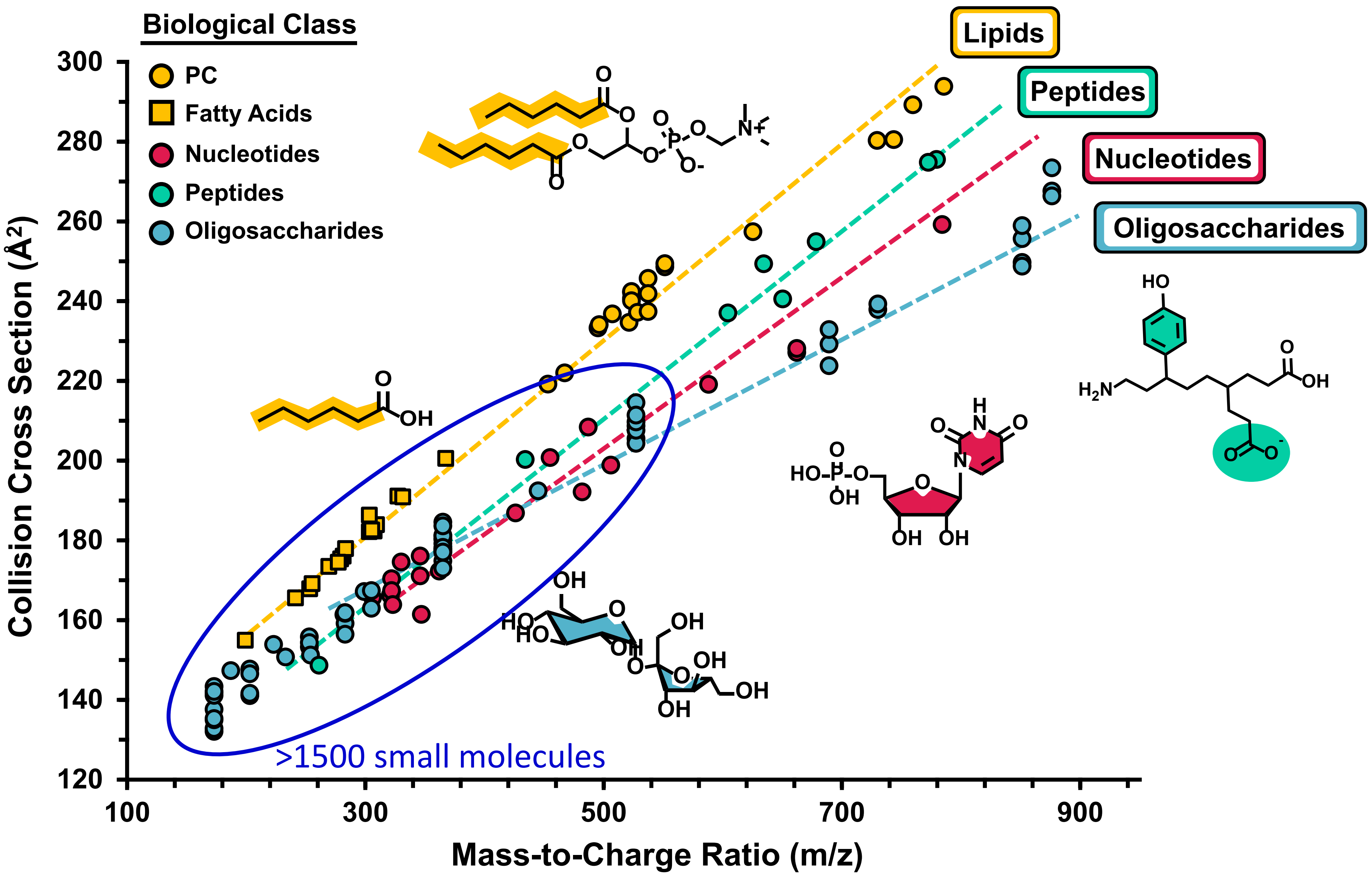
Butler, K.E., et al. *Anal. Bioanal. Chem.* 2021, 414, 623.

IMS & MS Trend Line Relationships



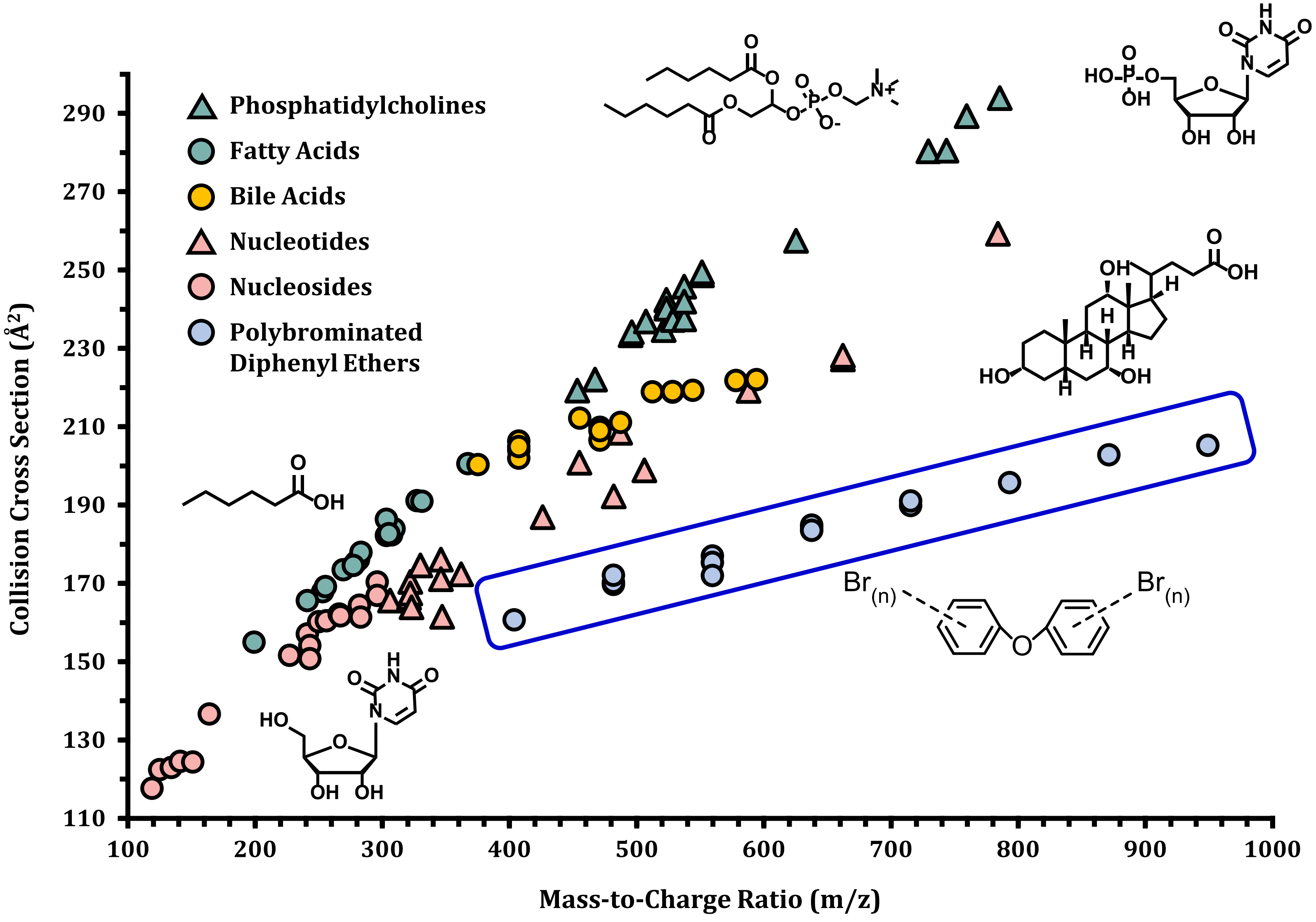
Fenn, L.S.; McLean, J.A. *Anal. Bioanal. Chem.* 2008, 391, 905.

IMS & MS Trend Line Relationships



Zheng, X., et al. *Chem. Sci.* 2017, 8, 7724.

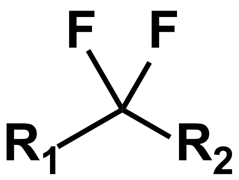
IMS & MS Trend Line Relationships



Burnum-Johnson, K.E., et al. *Trends Anal. Chem.* 2019, 116, 292.

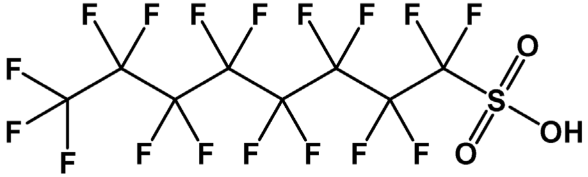
What are PFAS?

Per- and Polyfluoroalkyl Substances (PFAS)

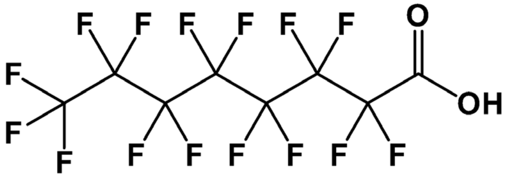


- Man-made chemicals
- Over 14,000 known PFAS
- Chemically inert
- Thermally stable

Legacy PFAS



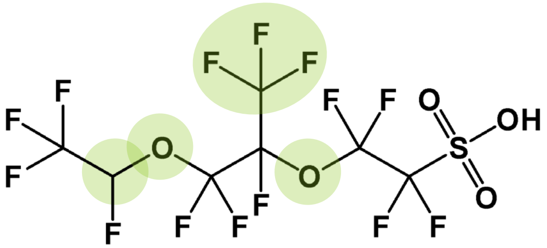
Perfluorooctanesulfonic acid (PFOS)



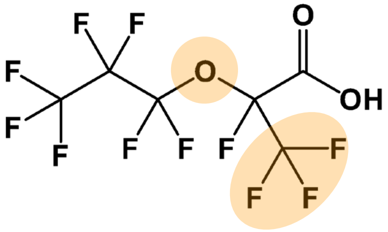
Perfluorooctanoic acid (PFOA)

Environmental and health concerns

Emerging PFAS



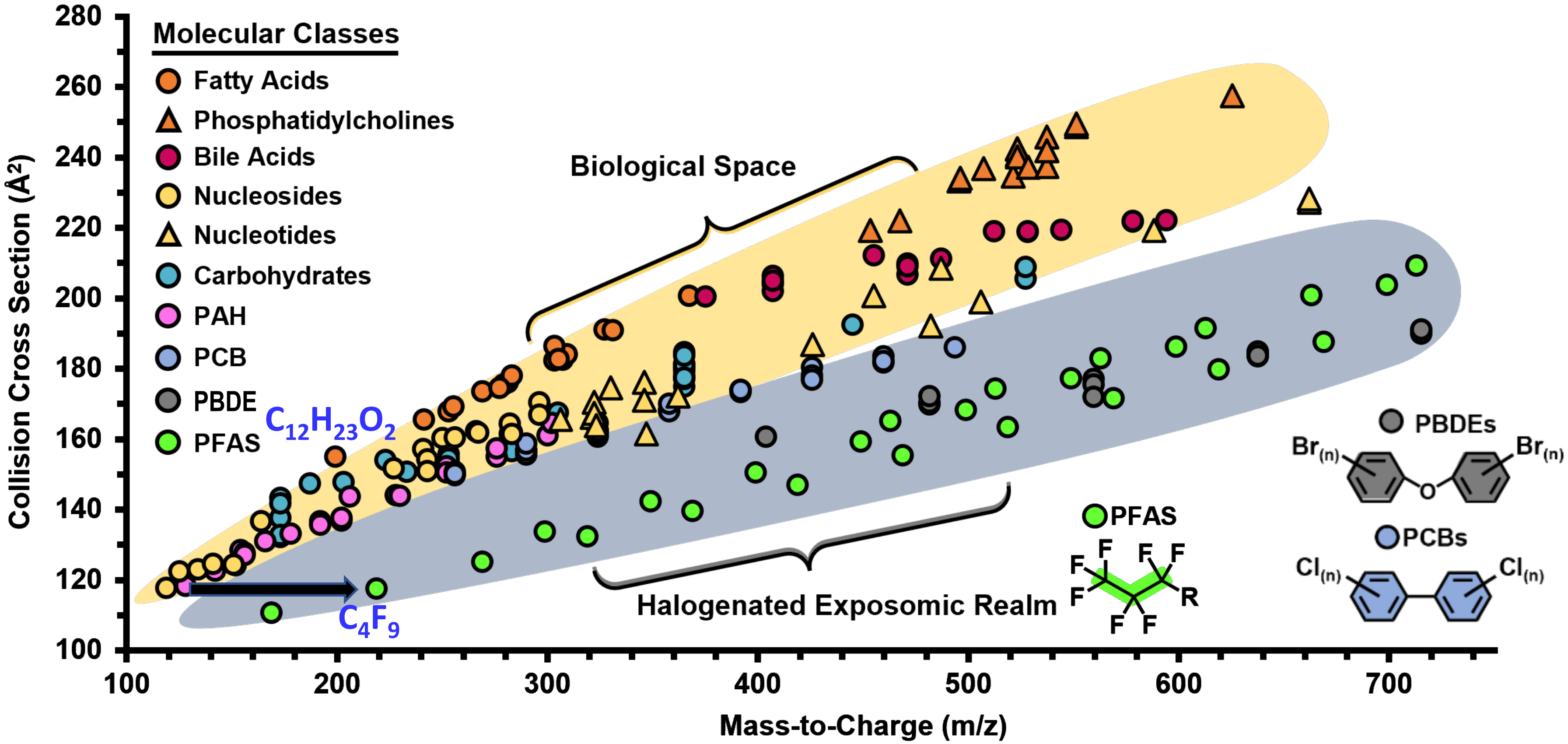
Nafion Byproduct 2



“GenX”

OECD “Portal on Per and Poly Fluorinated Chemicals”. 2023.
US EPA CompTox Chemicals Dashboard PFAS Master List. 2023.

IMS & MS Trend Line Relationships: Application to Chemical Exposome



Foster, M., et al. *Environ. Sci. Technol.* 2022. 56, 12, 9133.

Targeted and Non-Targeted Analyses of Environmental Exposome

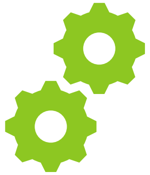
Targeted

- Limited to specific known analytes

Table 1. Current standards and consensus methods for PFAS analysis in the environment.

Method	Matrix Trend	No. of Analytes	Sample Prep Procedure	Quantification Technique
EPA 533	Drinking water	25	Solid phase extraction	Isotope dilution
EPA 537	Drinking water	14	Solid phase extraction	Internal standard correction
EPA 537.1	Drinking water	18	Solid phase extraction	Internal standard correction

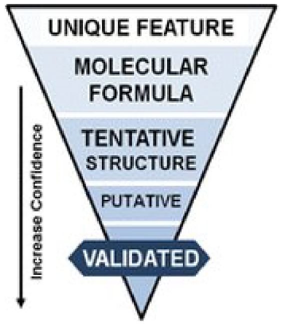
- Hypothesis testing



- Low resolution instruments
- Sensitive, quantitative, & rapid

Non-Targeted

- All features from a sample



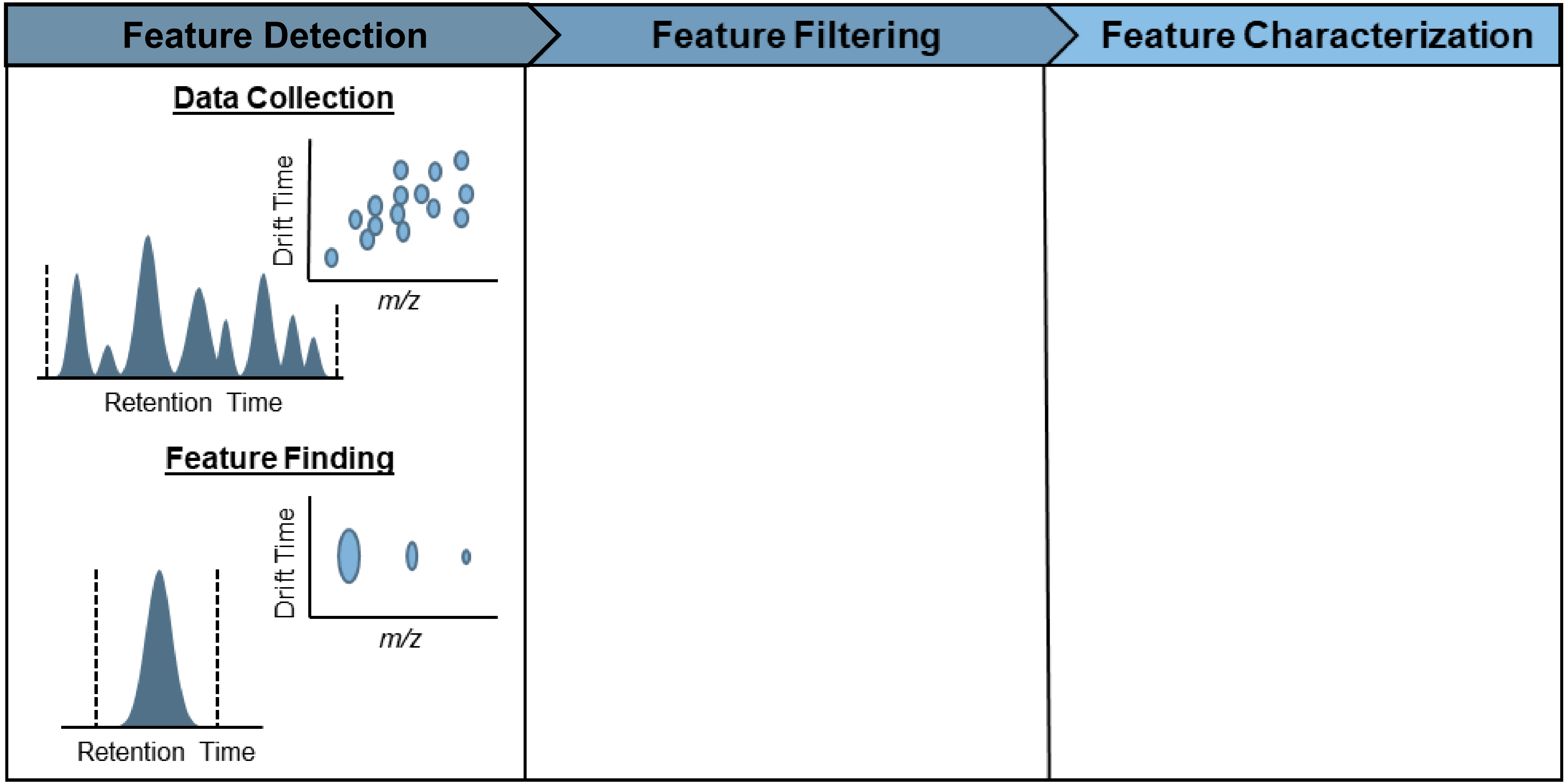
- Hypothesis generating

- Requires high resolution instrument
- Complex data and time-consuming

Non-targeted discoveries inform targeted studies

Schrimpe-Rutledge, A.C., et al. *J. Am. Soc. Mass Spectrom.* 2016, 27, 1897.

Non-Targeted PFAS Discovery Workflow



Donelson-Kirkwood K.I., et al. *Sci. Adv. 2023.* Accepted.
Roman-Hubers, A.T., et al. *Energy Fuels.* 2021, 35, 10529.

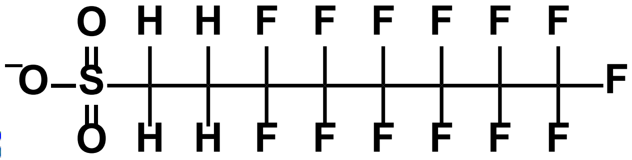
Fragment Drift Alignment and Signal Filtering

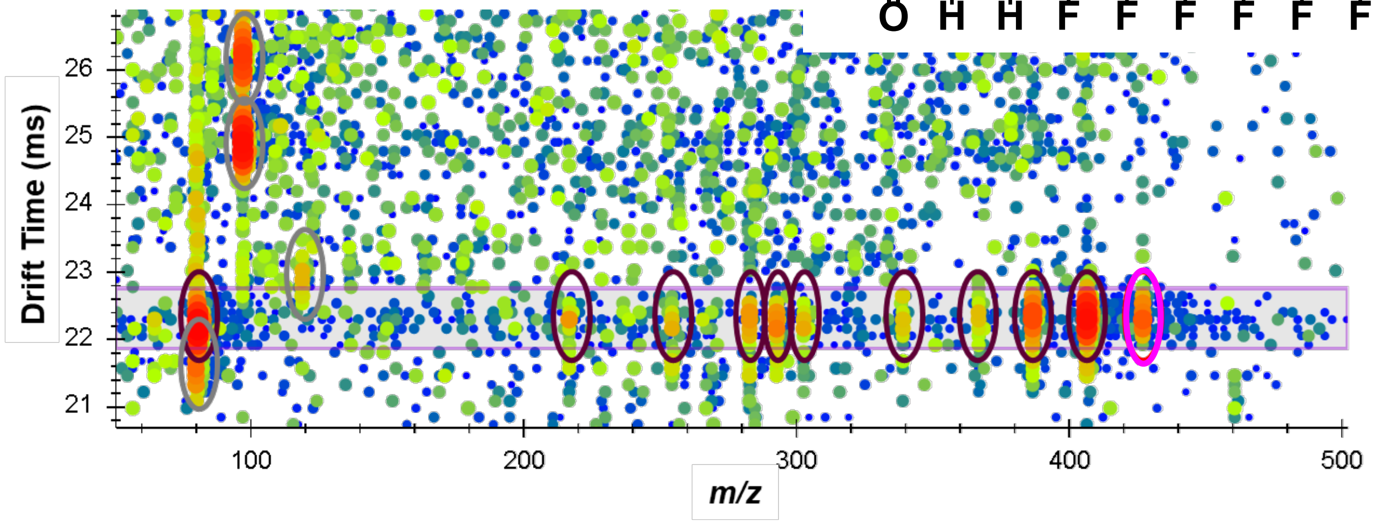
Precursor

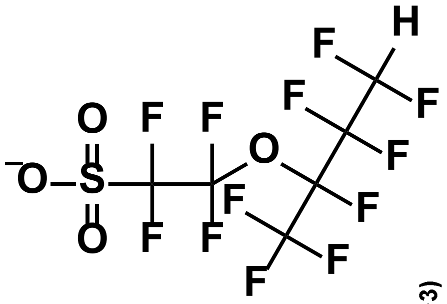
Drift-aligned fragments

Other fragments

6:2 FTS

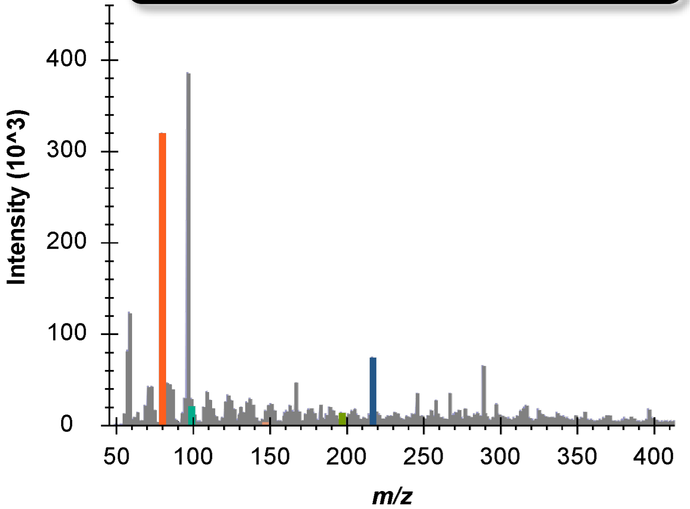


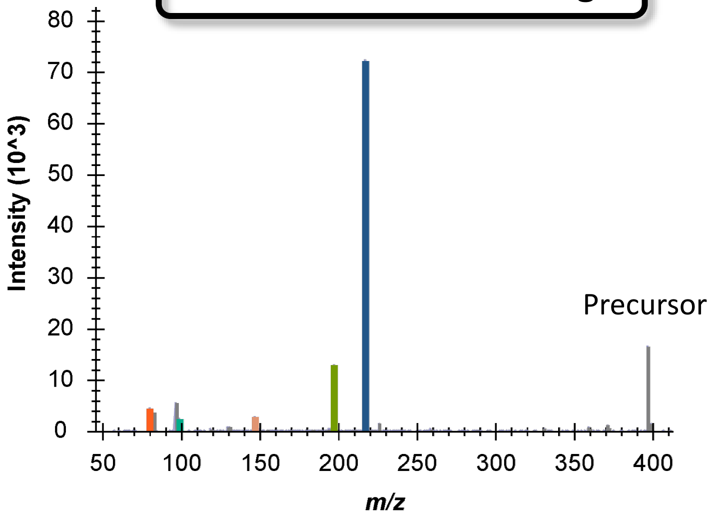




Nafion byproduct 6

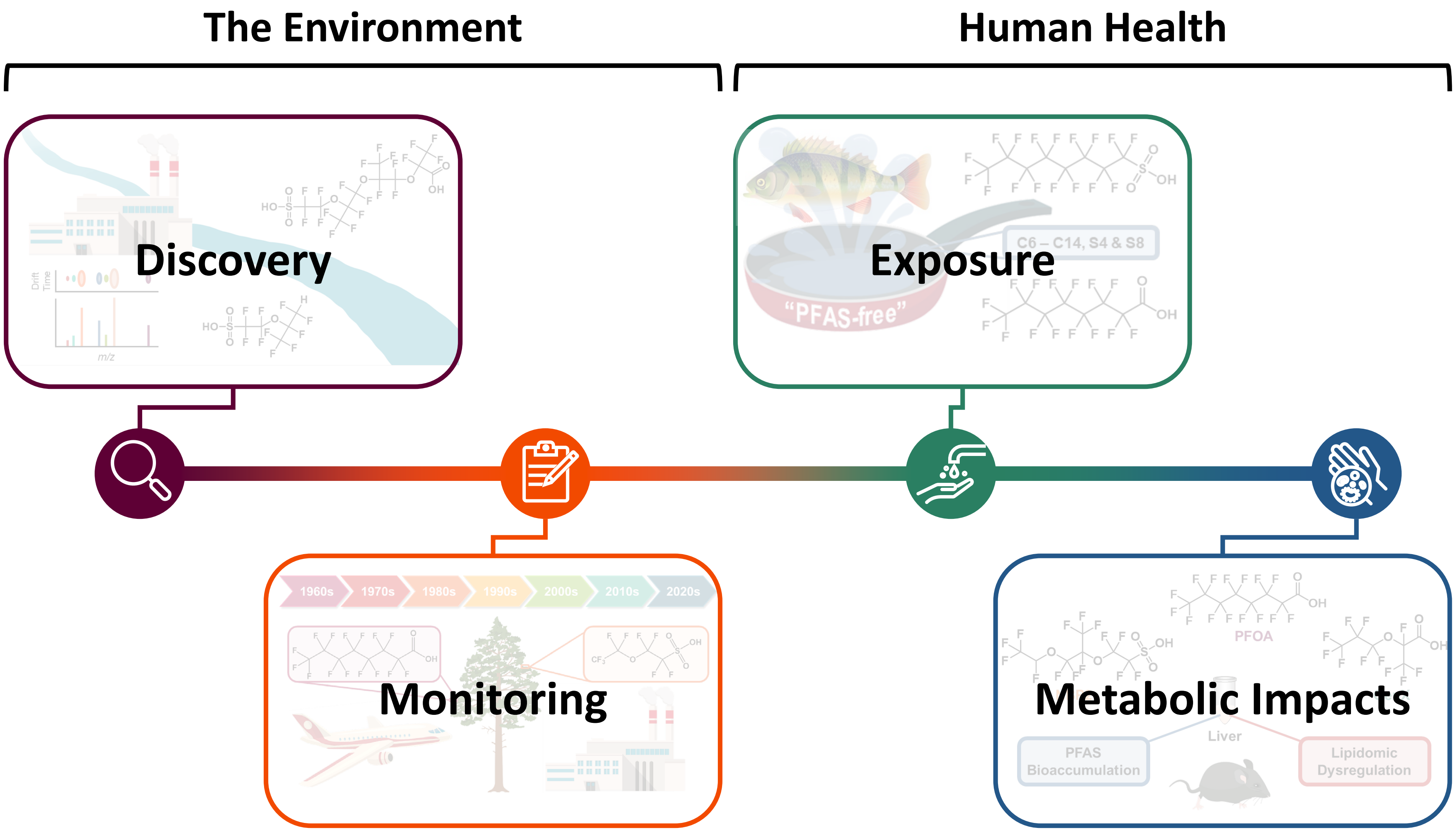
Without Drift Time Filtering → With Drift Time Filtering



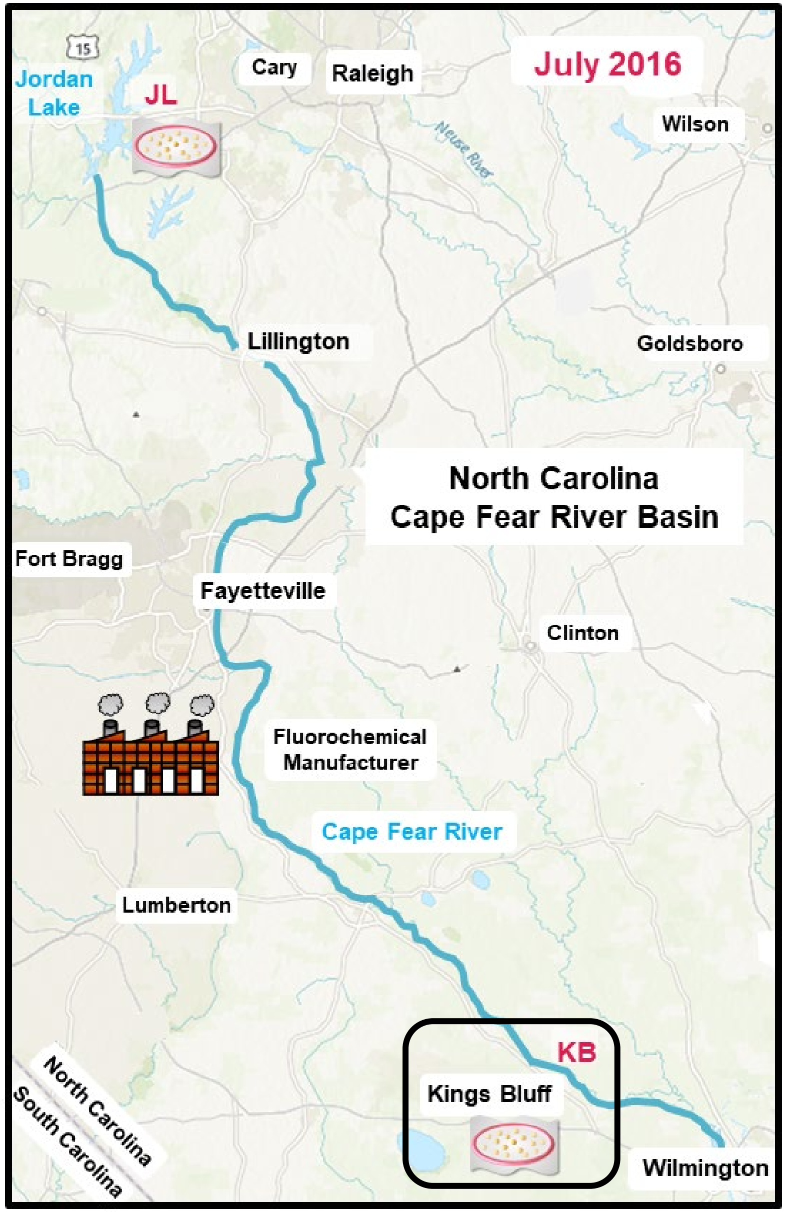


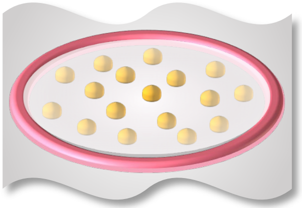
Non-Targeted Approach

- Using non-targeted multidimensional approach to study PFAS implications on…



Application Overview: Aquatic Passive Samplers





HP20 resin in nylon mesh
2-week deployment

57 fluorinated features

36 known | **21 unknown**

↓

14 unique molecular formulas

↓

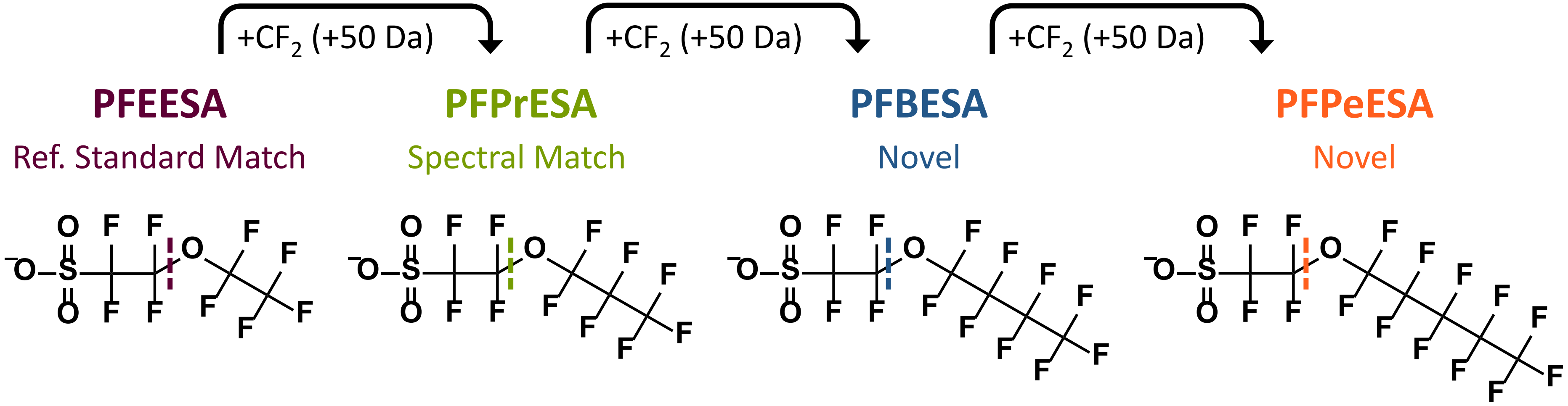
11 proposed structures

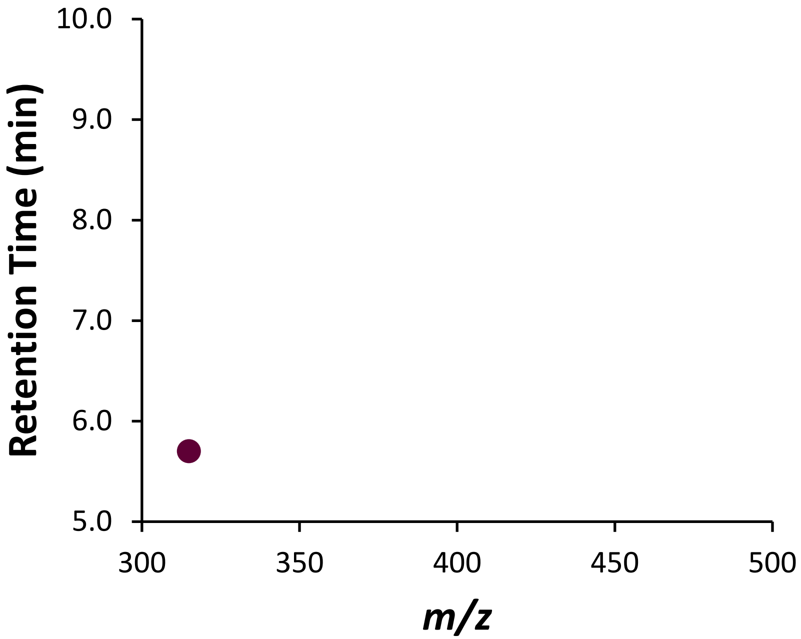
Astrid Schnetzer
NCSU

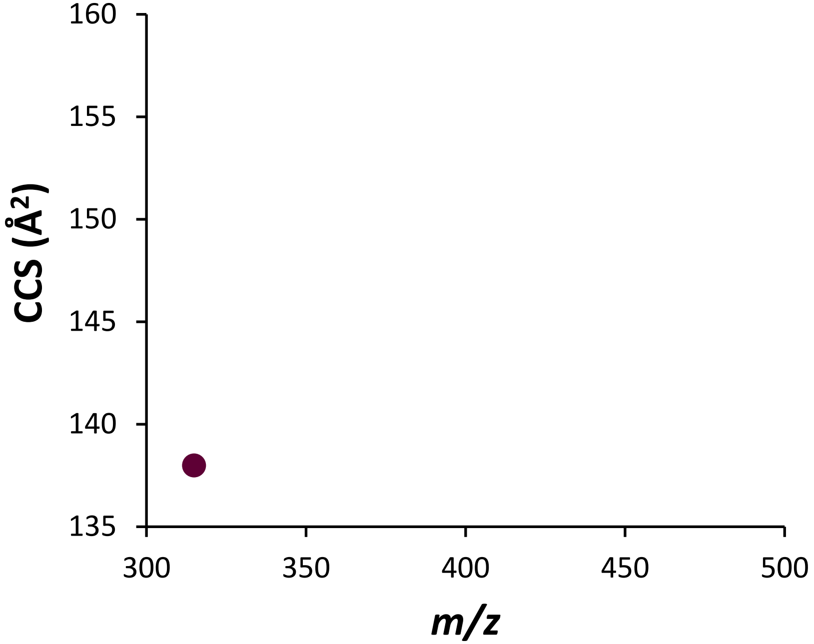
Kaylie Kirkwood

Kirkwood-Donelson, K.I., et al. *Sci. Adv. 2023,* Accepted.

Homologous Series Example



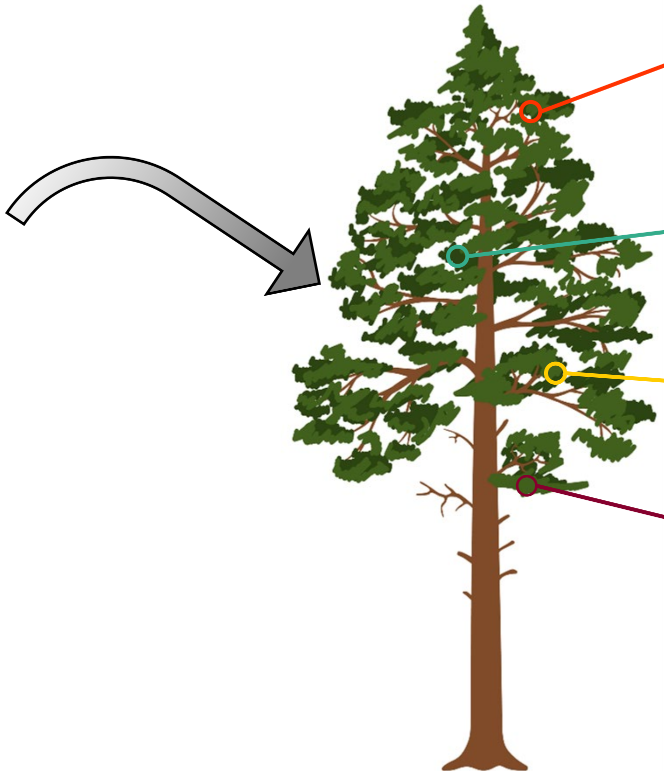




Kirkwood-Donelson, K.I., et al. *Sci. Adv. 2023,* Accepted.
McCord, J. et al. *Environ. Sci. Technol.* 2019, 53.

Pine Needles as Passive Samplers

Pesticides
PCBs
PAHs
PBDEs
Metals
PFAS

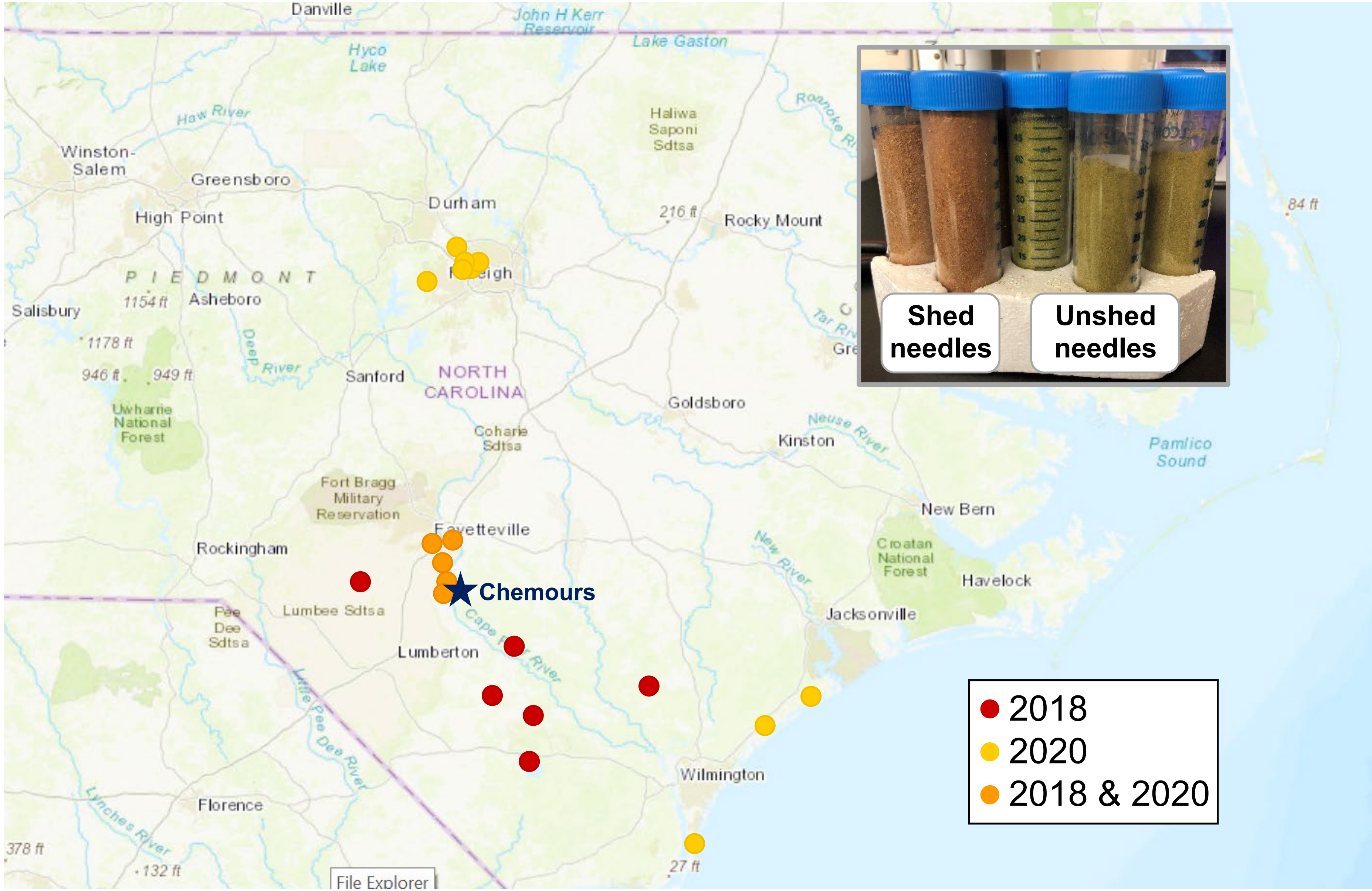


- Needles have strongly adsorptive wax cuticles
- Needles are easy to collect and store
- Needles drop regularly for temporal studies
- Fewer changes in water content than broad-leaved trees

Question: Can we use pine needles to monitor the spread of legacy AND emerging PFAS in NC?

Zhang, H., et al. *Environ. Toxicol. Chem.* 2015, 34, 1499.
Chropeňová, M., et al. *Environ. Sci. Technol.* 2016, 50, 9487.
Kirkwood, K.I., et al. *Environ. Sci. Technol.* 2022, 56, 3441.

Site Sampling

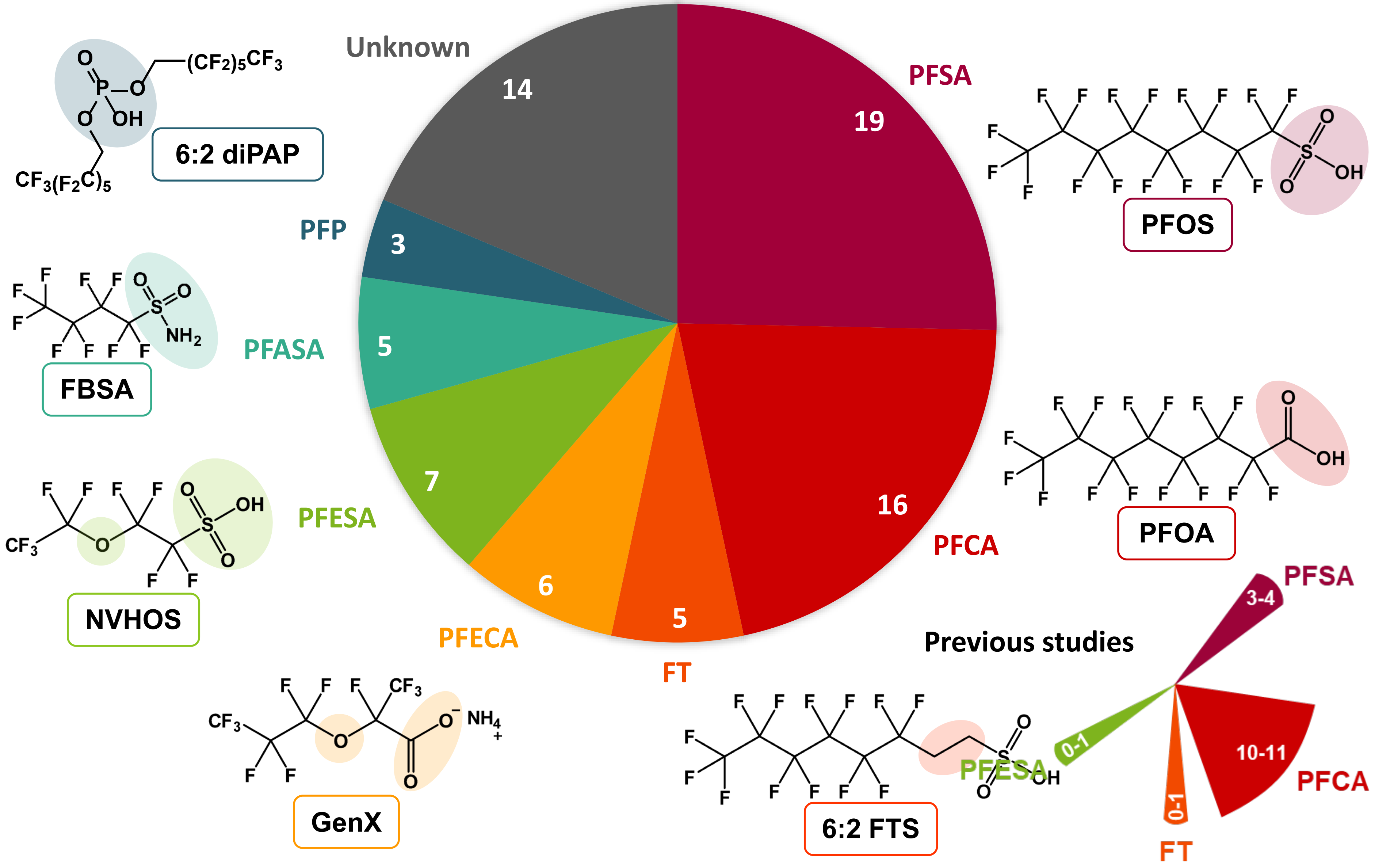


Scott Belcher
NCSU

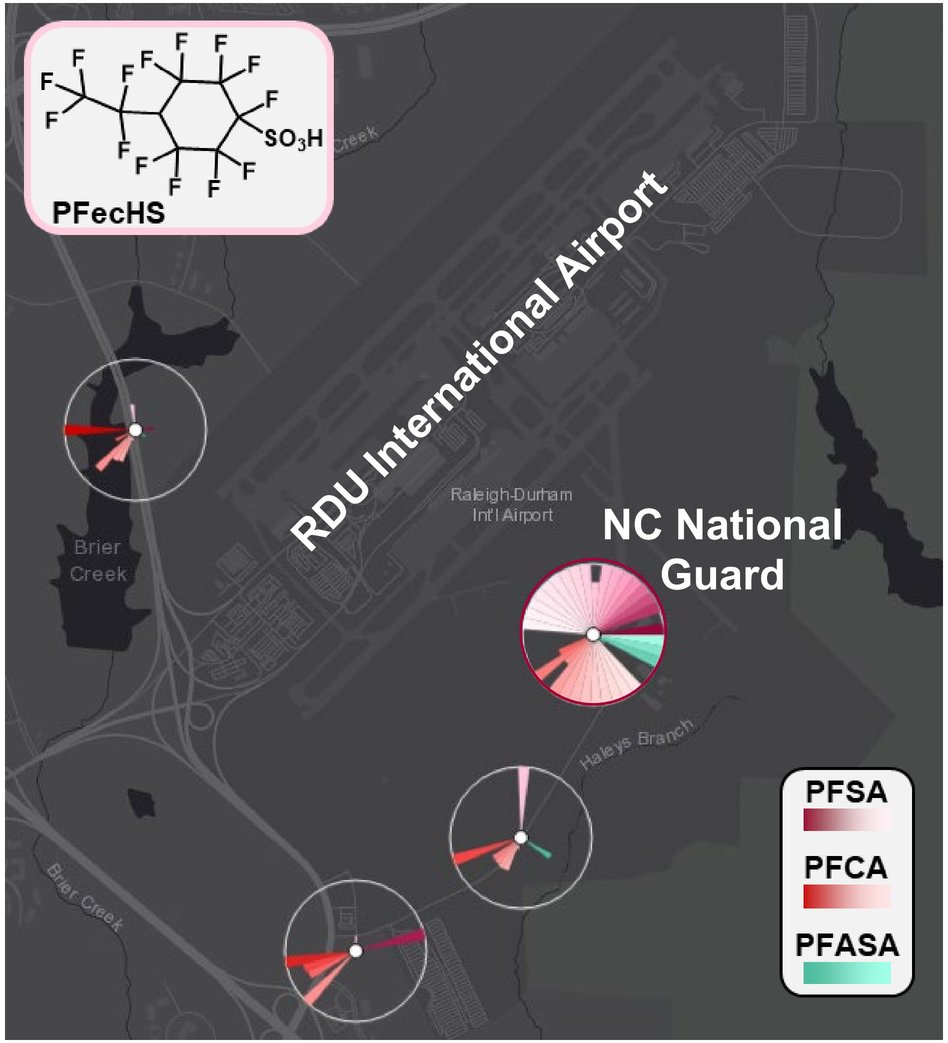
Kirkwood, K.I., et al. *Environ. Sci. Technol.* 2022, *56*, 3441-3451.

Detected PFAS in Pine Needles

- Using library with >100 PFAS *m/z*, retention time and CCS information from standards



Other Point Sources Found from Pine Needles

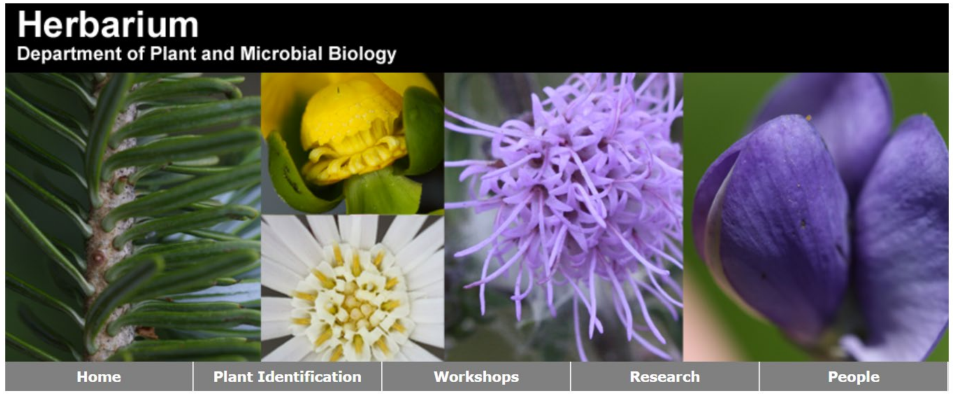


- 2-4 orders of magnitude higher concentrations of linear and branched C4-C8 PFSAs & PFCAs

Kirkwood, K.I., et al. *Environ. Sci. Technol.* 2022, *56*, 3441-3451.

Historical Analysis of Archived Needles

North Carolina State University Vascular Plant Herbarium (NCSC) est. 1898

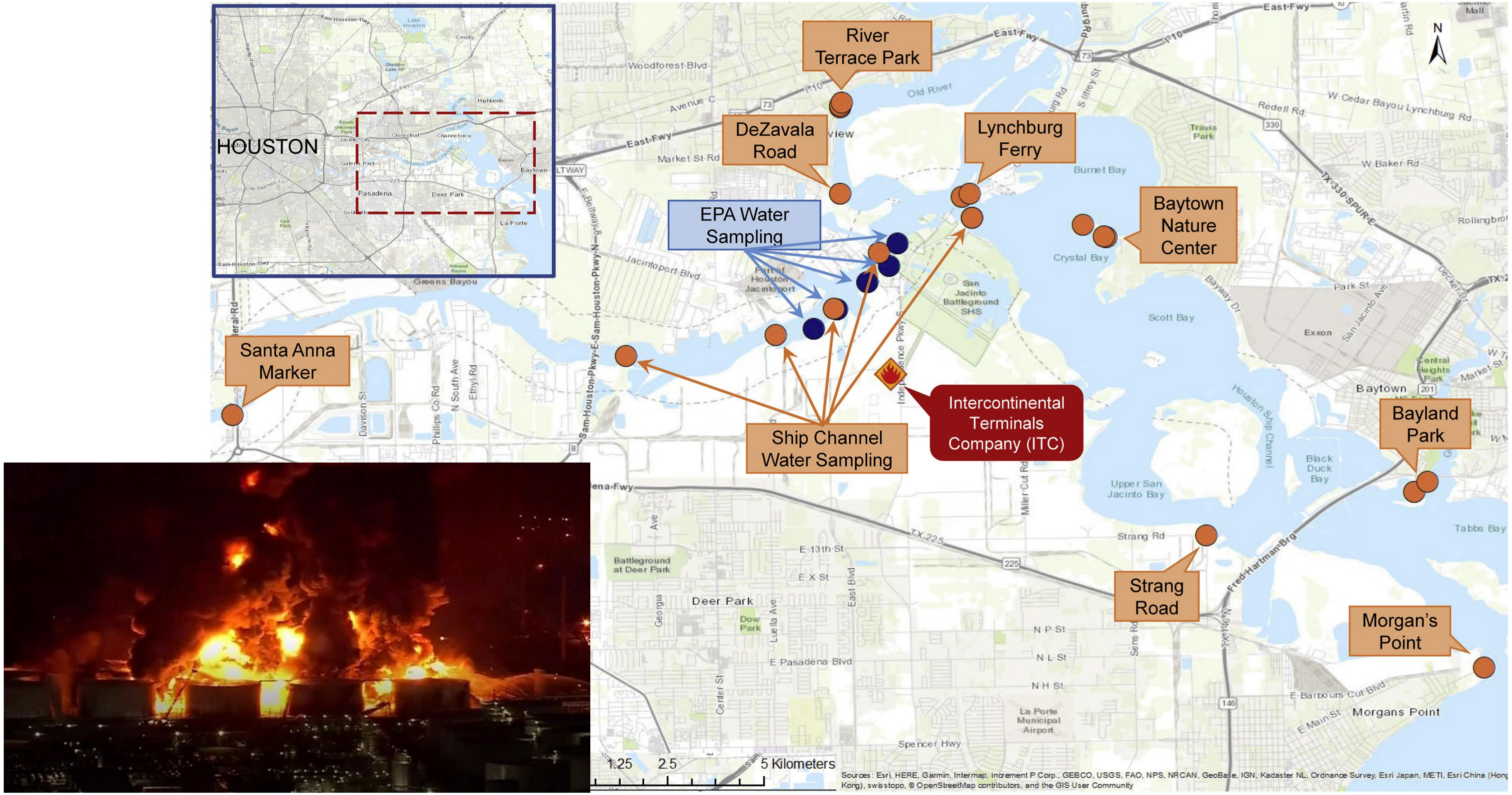


Duke University Herbarium est. 1921

Plant species
Location description
Date of collection

Intercontinental Terminals Company (ITC) Fire Water Analyses

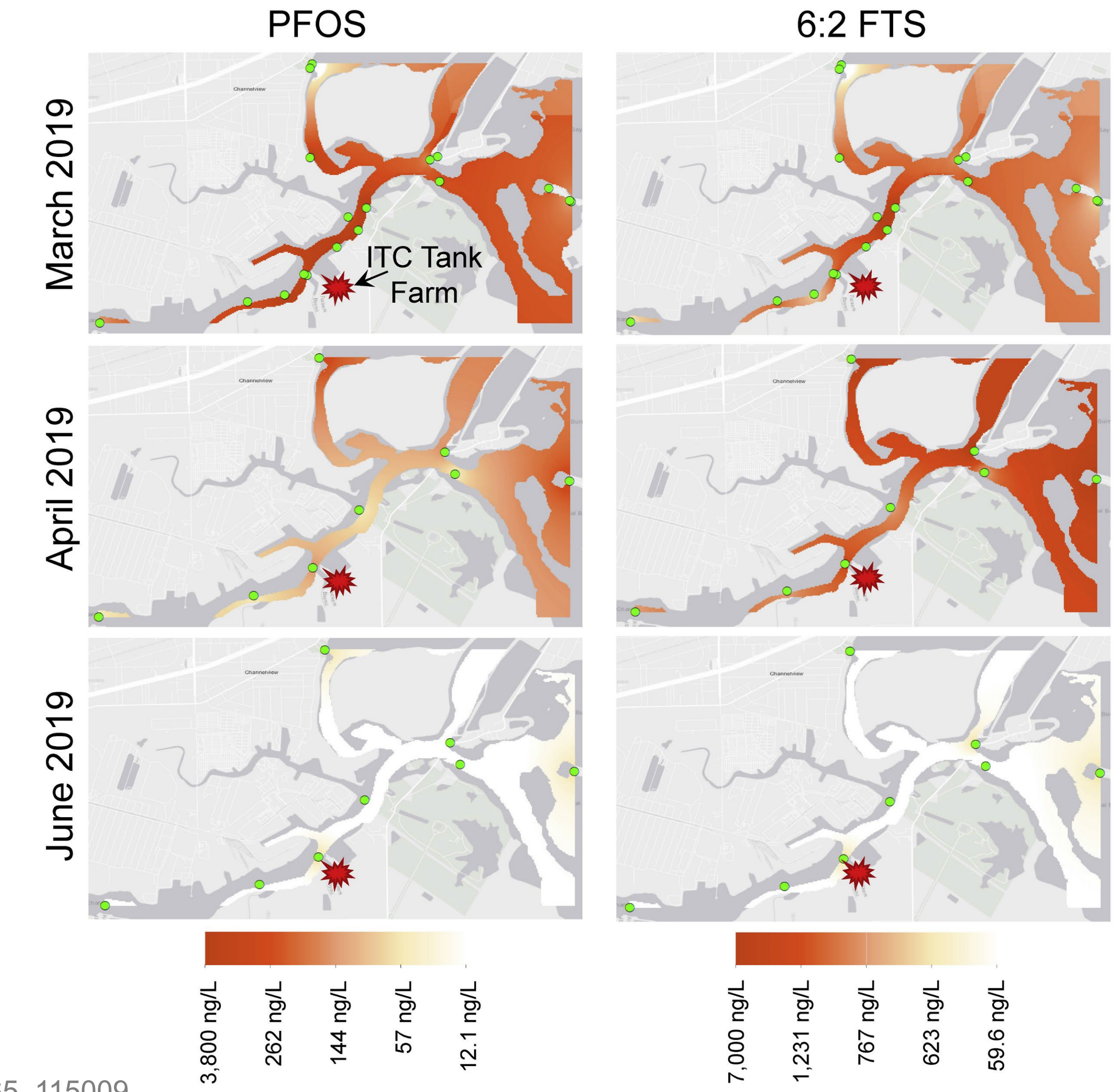
- Large-scale fires at ITC in Houston where ~5 million liters of class B firefighting foams were used



Ivan Rusyn
Texas A&M

Aly, N.A., et al. *Environ. Pollut.* 2020, 265, 115009.

Intercontinental Terminals Company (ITC) Fire Water Analyses



Aly, N.A., et al. *Environ. Pollut.* 2020, 265, 115009.
Luo, Y.S., et al. *Environ. Sci. Technol.* 2020, 54, 15024.

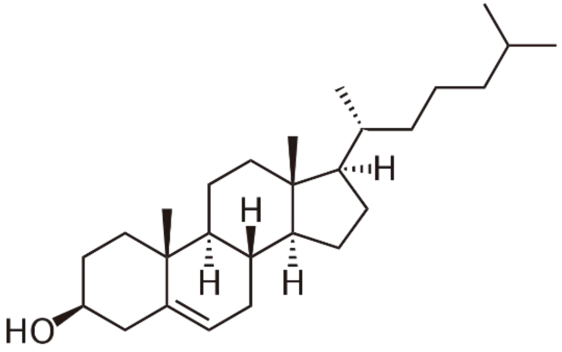
Exposure and Lipid Analyses

Exposure

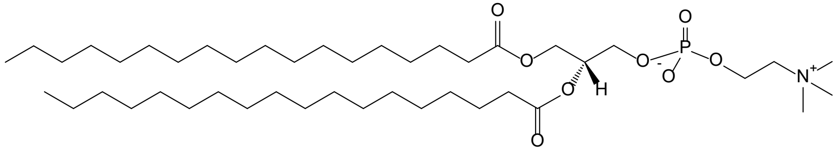
↓ **?**

Health Outcomes

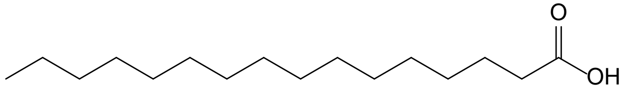
"Increased cholesterol levels among exposed populations"

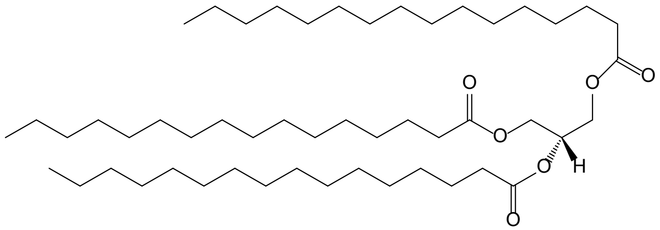


Link exposure to suppression of genes involved in fatty acid metabolism



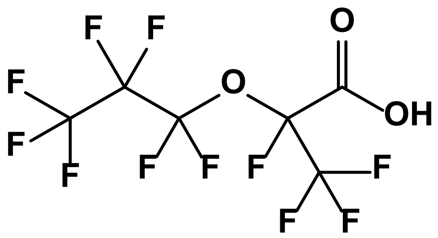
Higher risk of incident hypertriglyceridemia





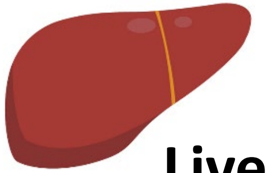
US EPA. PFOA & PFOS fact sheet. 2017.
Lin, P.D. et al. *Environ. Int.* 2019, 129, 343.
Droge, S.T.J. *Environ. Sci. Technol.* 2019, 53, 760.
Jacobsen, A.V. et al. *Environ. Sci. Pollut. Res. Int.* 2018, *25, 23074*.

Evaluating Hepatic PFAS Accumulation



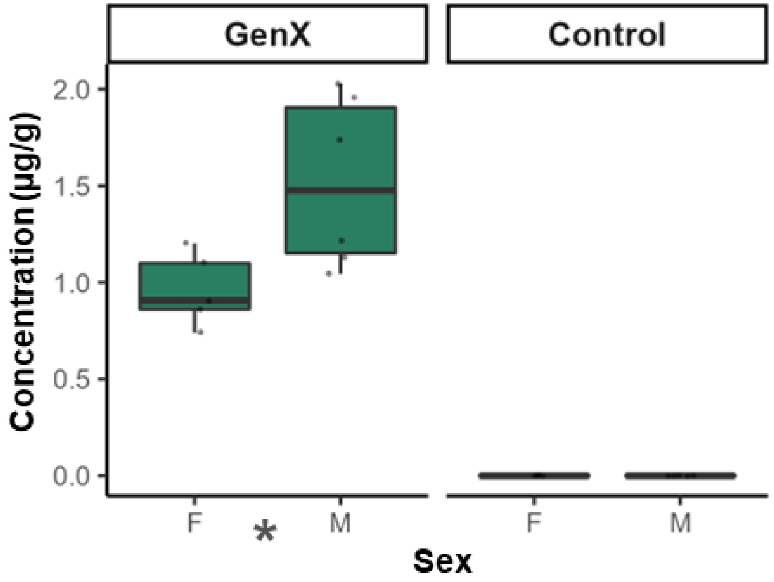
GenX
100 mg/kg/day

Control = 19
Exposed = 11



Liver

419 detected lipids
123 significantly altered lipids

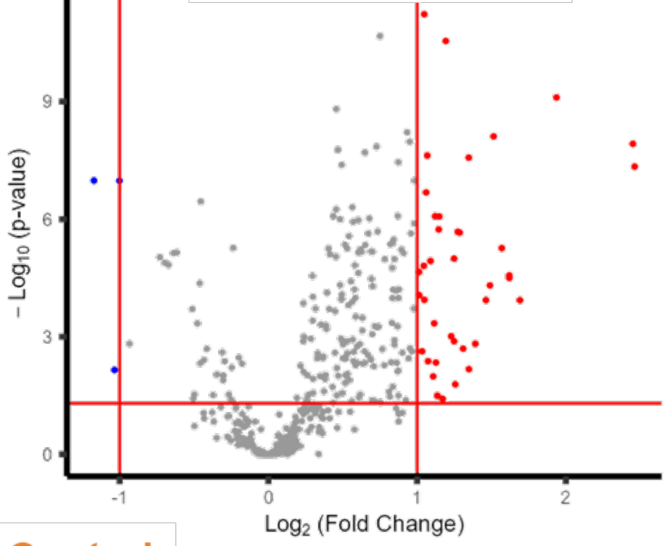


Jamie DeWitt
OSU

DeWitt, J.C. et al. *Environ. Health Perspec.* 2008, 116, 644.
McDonough, C.A. et al. *Environ. Sci. Technol.* 2020, 54, 5700.

Lipid Upregulation Following PFAS Exposure

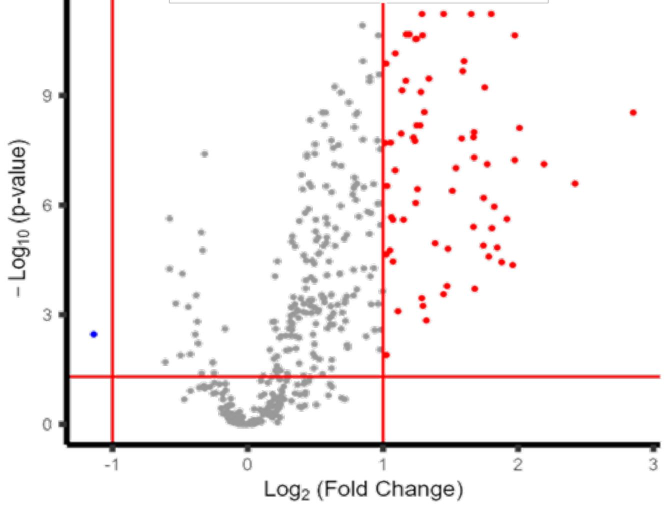
PFOA vs. Control



123 significantly altered lipids
7 downregulated
116 upregulated

$p < 0.05$
$\log_2 FC > 1$

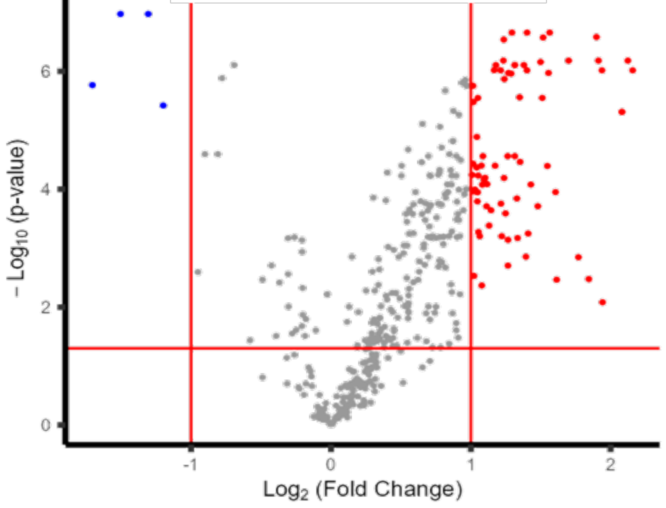
NBP2 vs. Control



Lipid accumulation

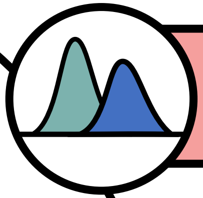
Liver enlargement

GenX vs. Control



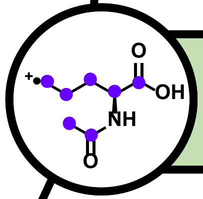
Conley, J.M. et al. *Environ. Int*. 2022, 170, 107631.
Schlezinger, J.J. et al. *Toxicol. Appl. Pharmacol.* 2021, 426, 115644.

Conclusions



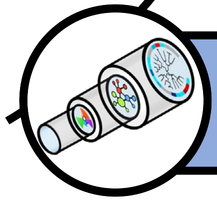
Ion Mobility Spectrometry

- Removes interferences
- Increases -omic ID confidence
- Enables rapid analysis



Molecular Libraries

- Experimental and theoretical CCS



Non-Targeted Screening

- Ability to assess known and unknown chemicals
- Identify targets for follow-up analyses

Future Opportunities:

- CCS libraries will enable the screening of thousands of samples per year
- Multidimensional non-targeted LC-IMS-MS analyses will detect novel chemicals

Acknowledgements

Baker Lab Members

Research Assistant Professor

- Prof. James Dodds

Postdoctoral Researcher

- Dr. Guozhi Zhang

Graduate Students

- Jack Ryan
- Anna Boatman
- Jessie Chappel
- Amie Burnell
- Ashlee Falls
- Greg Kudzin

Past Graduates with Significant Contributions

- Dr. Karen Butler
- Dr. Melanie Odenkirk
- Dr. Kaylie Kirkwood Donelson
- Dr. Nancy Lee Alexander

Sources of Funding

- NIEHS P42 ES027704 & P42 ES031009
- NIGMS RM1 GM145416 & R01 GM141277
- US EPA
- NC WRRI

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Collaborators

Texas A&M
Rusyn Group
Johnson Group
Chiu Group
Laganowsky Group

NCSU
Belcher Group
Knappe Group
Patisaul Group
David Reif
Casey Theriot
Owen Duckworth

PNNL
Kelly Stratton
Kristin Burnum-Johnson

U Washington
Mike MacCoss
Brendan MacLean
Skyline Guys

Oregon State U
Jamie DeWitt

U Arizona
Marty Group

Georgia Tech
Fernández Group

Scripps
Siuzdak Group

UTMB
Brandie Taylor

NIST
John Schiel

Emory
Blaine Roberts

UCSD
Dorrestein Group