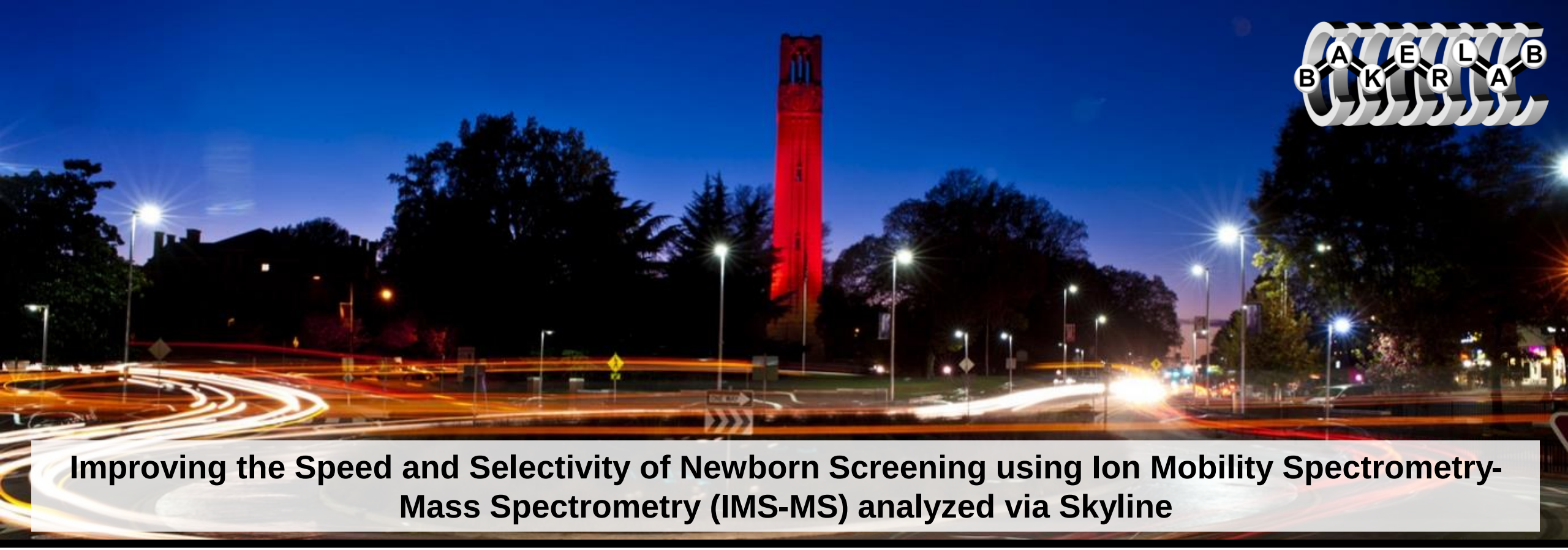


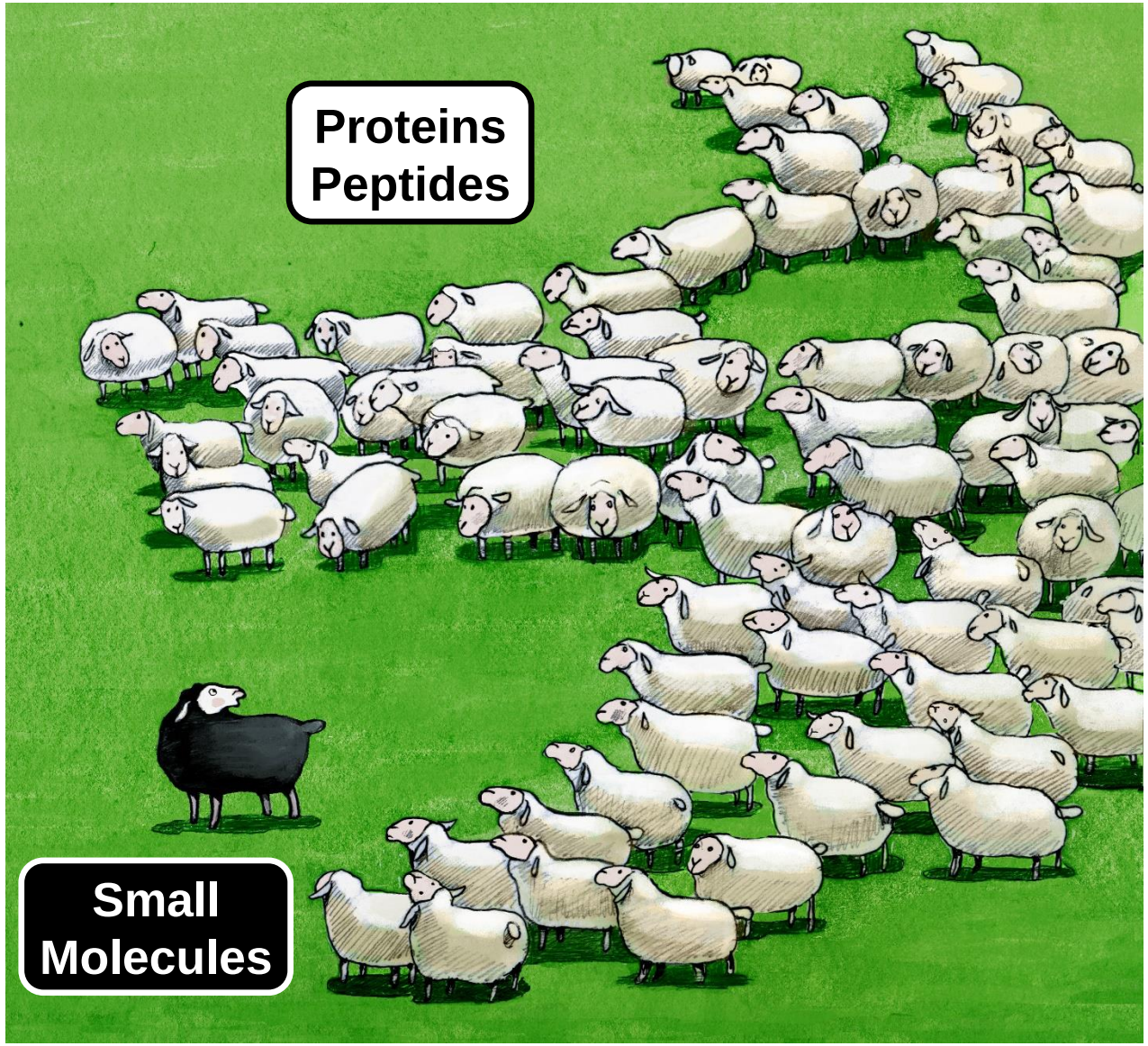
Improving the Speed and Selectivity of Newborn Screening using Ion Mobility Spectrometry-Mass Spectrometry (IMS-MS) analyzed via Skyline

James Dodds – Erin Baker's Lab

11th Annual Skyline User Group Meeting

Sunday, June 5, 2022

11th Annual Skyline User Group Meeting



Baker Lab Research with Skyline

Exposomics ~ Pesticides, PFAS

Kaylie Kirkwood
WOH am

Nancy Lee Alexander
MOH pm

Anna Boatman
MP 138

Proteins

Jack Ryan
MP 476

Bile Acids

Allison Stewart

Prof. Erin Baker

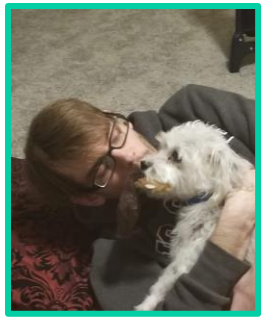
Lipidomics ~ Lipid Dysregulation

Dr. Melanie
Odenkirk

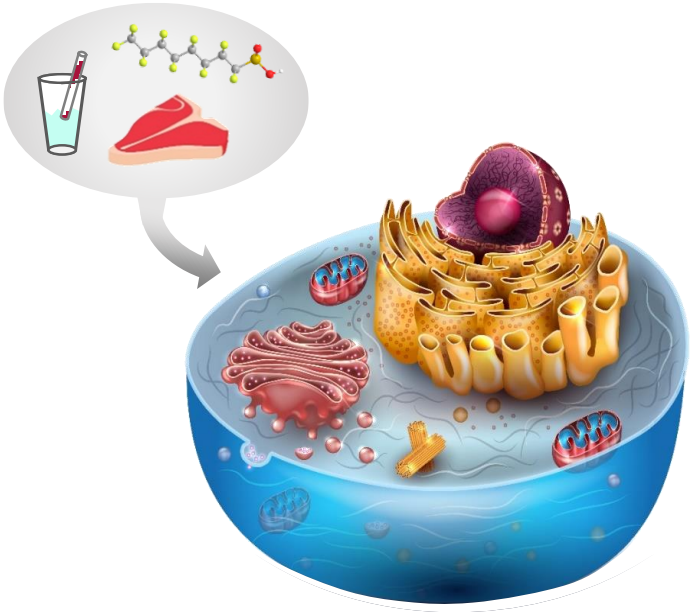
Rebecca Beres
WOF am

Jessie Chappel
WP 304

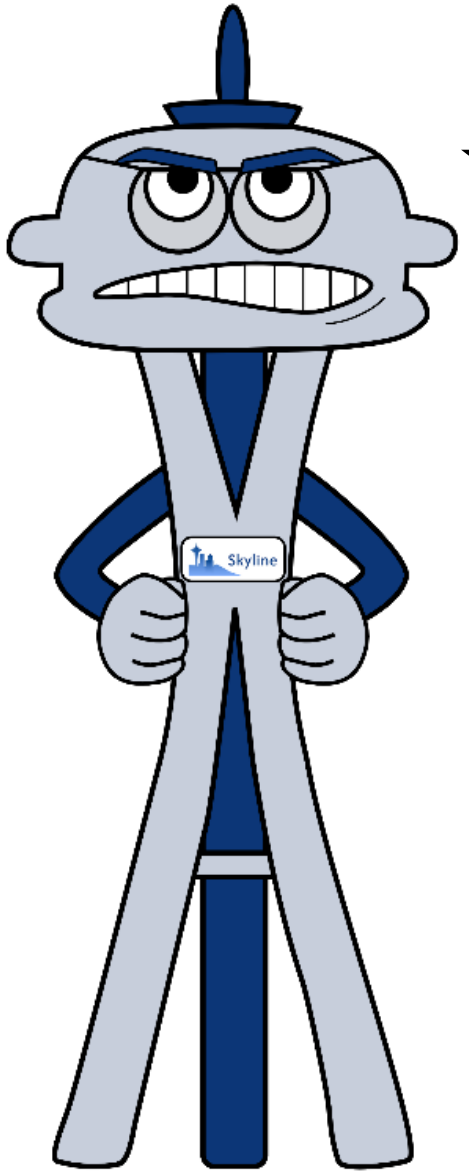
Metabolomics



Me and Mugsy
TP 084



Baker Lab and Skyline Publications



1. MacLean, B.X. *et. al.* “Using Skyline to Analyze Data-Containing Liquid Chromatography, Ion Mobility Spectrometry, and Mass Spectrometry Dimensions.” *J. Am. Soc. Mass Spectrom.* **2018**, *29*, 2182-2188.

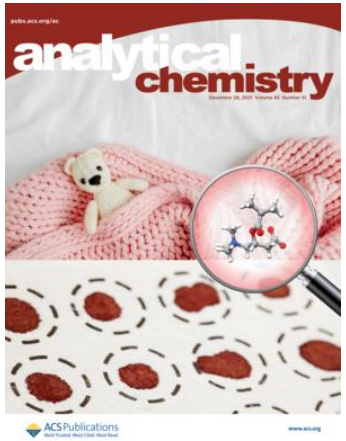
★ **2.** Dodds, J. N. *et. al.* “Improving the Speed and Selectivity of Newborn Screening Using Ion Mobility Spectrometry-Mass Spectrometry.” *Anal. Chem.* **2021**, *93*, 17094-17102.

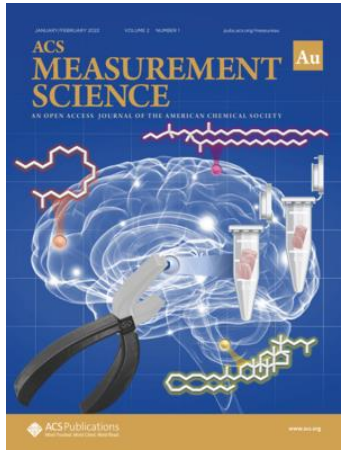
3. Kirkwood, K. I. *et. al.* “Utilizing Pine Needles to Temporally and Spatially Profile Per- and Polyfluoroalkyl Substances (PFAS).” *Environ. Sci. Technol.* **2022**, *56*, 3441-3451.

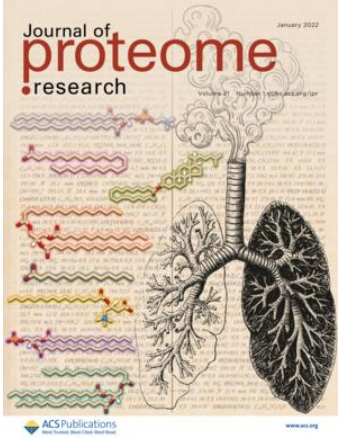
4. Kirkwood, K. I. *et. al.* “Development and Application of Multidimensional Lipid Libraries to Investigate Lipidomic Dysregulation related to Smoke Inhalation Injury Severity.” *J. Proteome Res.* **2022**, *21*, 232-242.

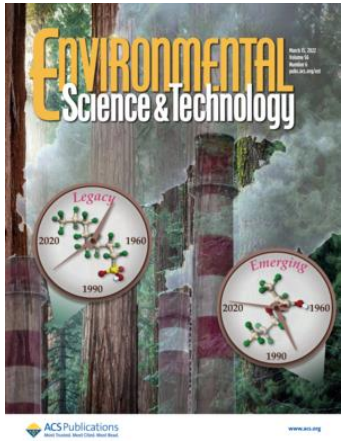
5. Odenkirk, M. T. *et. al.* “Combining Micropunch Histology and Multidimensional Lipidomic Measurements for In-Depth Tissue Mapping.” *ACS Meas. Sci. Au.* **2022**, *2*, 67-75.

6. Butler, K. E. *et. al.* “High-Resolution Demultiplexing (HRdm) Ion Mobility Spectrometry-Mass Spectrometry for Aspartic and Isoaspartic Acid Determination and Screening.” *Anal. Chem.* **2022**, *94*, 6191-6199.



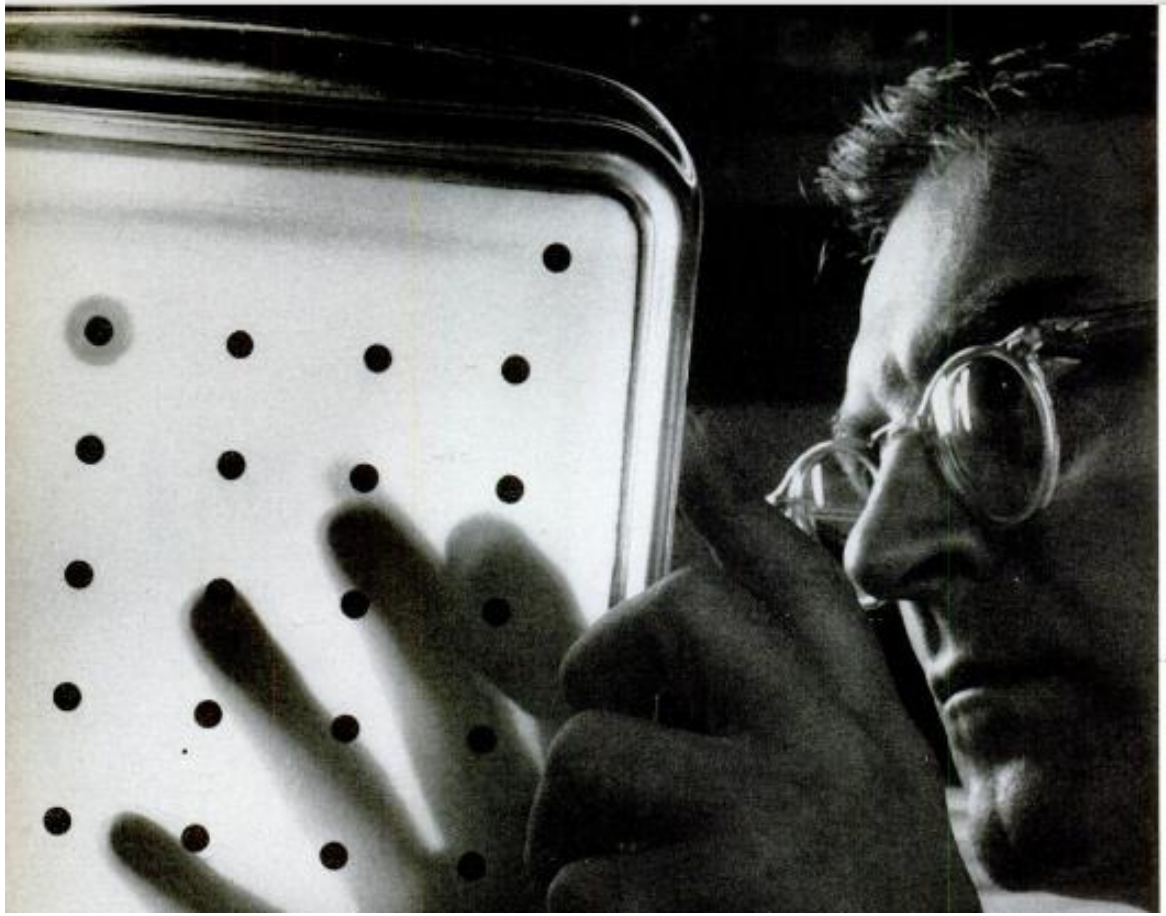






Newborn Screening (NBS)

Congenital Disorders (aka “Conditions**)**
Genetic, Enzyme deficiencies



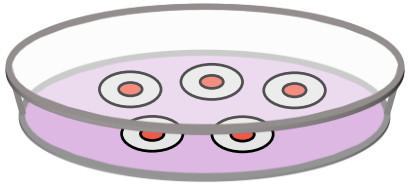
DISEASE DETECTOR
CONTINUED

New test for PKU is demonstrated by its discoverer, Dr. Robert Guthrie, at Children's Hospital in Buffalo, N.Y. Blood, drawn from babies' heels, is placed on labeled pieces of absorbent filter paper. Tiny disks are punched from papers and set on the surface of a glass dish coated with special nutrient and a culture of bacteria. The bacteria will multiply only if phenylalanine is present on the paper disks. Most of the blood disks in the dish shown here are normal. But the one in the top row, second from the left, is surrounded by a halo of bacteria which reveals the presence of phenylalanine.

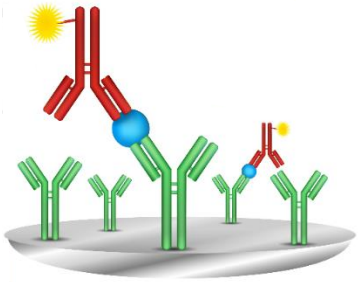
Phenylketonuria (PKU)

“PKU Test” – Robert Guthrie (1960s)

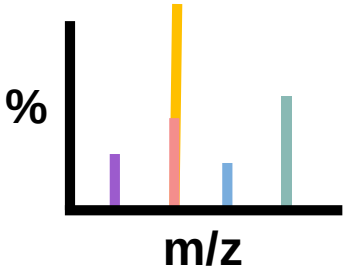
Bacteria Inhibition Assay



Immunoassay



Mass Spectrometry (FIA-MS/MS**)**



PKU ~ PHE/TYR

1) *Life* Magazine, Jan 19, **1962**, *52*, No. 3. ISSN: 0024-3019. “A Wintertime Special: Excitement on the Ice.” p. 45-46.

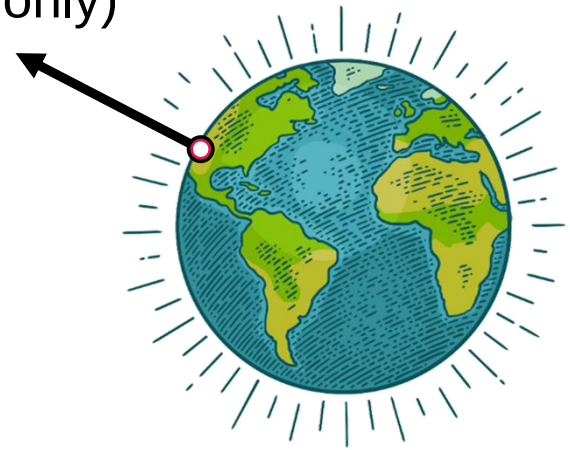
Newborn Screening (NBS)

~ 61 conditions recommended
~ 30-70 based on state

Tennessee (67)

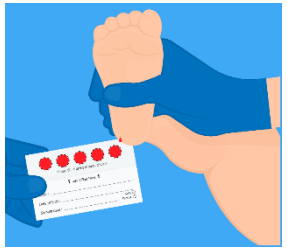
Connecticut (73)

~ 3.8 million / year
(US only)



1-2
Day/old

Heel Prick
(DBS)



Mail to
Testing Facility

Analytical Assays

MS/MS, Immunoassay

1) Janzen, N. et. al. *J. Clin Endocrinol. Metab.* **2007**, *92*, 2581-2589. https://doi.org/10.1210/jc.2006-2890

1) https://newbornscreening.hrsa.gov/your-state/

Analytical Challenges

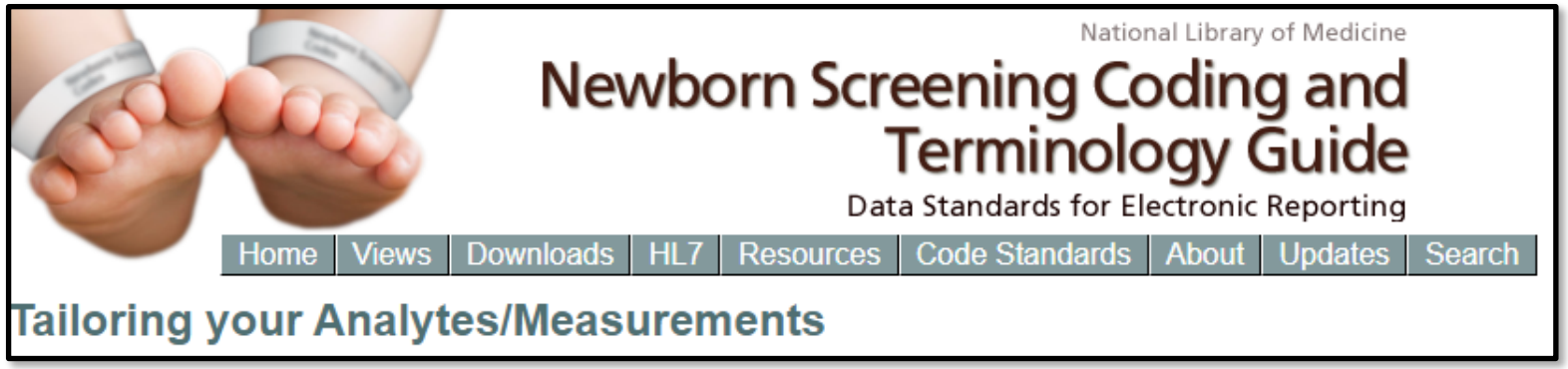
~ Millions of samples

~ Time constraints

~ Need screening and confirmation (1st and 2nd tier testing)

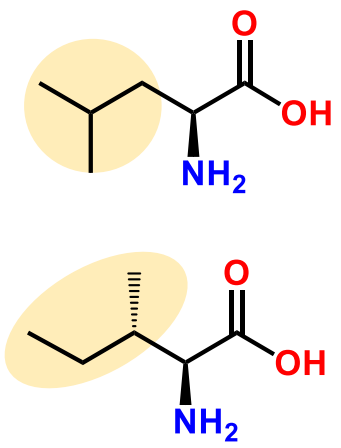
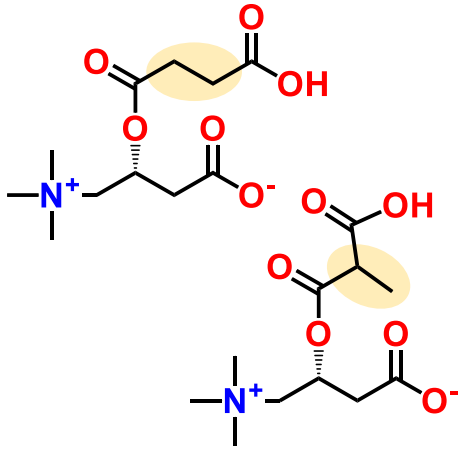
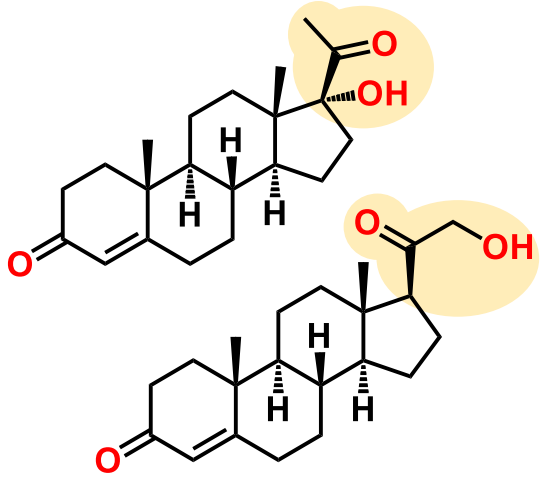
~ Chemical and isomeric diversity

~ 3.8 million infants
(US only)



~ 70 molecules including amino acids, acylcarnitines, hormones etc.

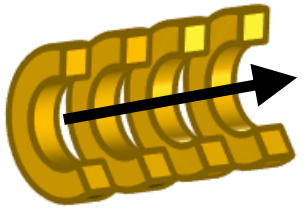
Molecular Challenges in NBS

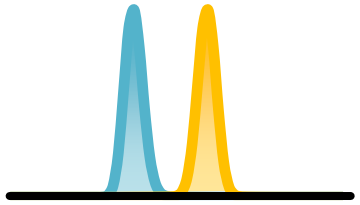
Amino Acid Isomers	Carnitine Isomers	Hormone Isomers
		
Leucine / Isoleucine	Succinyl / Methylmalonyl	17α-OHP / DOC

Project Goals

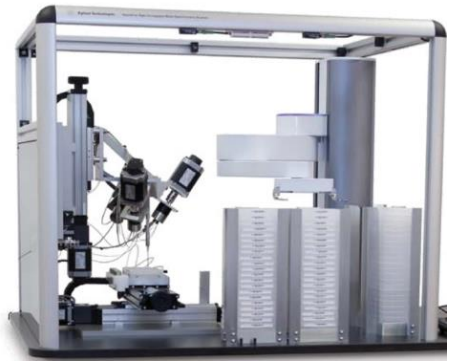
1) **Improve Selectivity** – Isomer separation by IMS

2) **Increase Throughput** – Automated SPE extraction using Agilent RapidFire
(~ 10 sec/sample – sample vs. 2 mins)



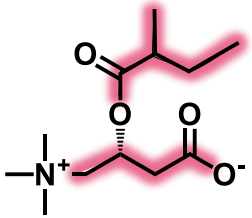


Agilent RapidFire

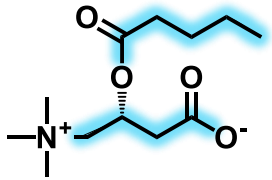


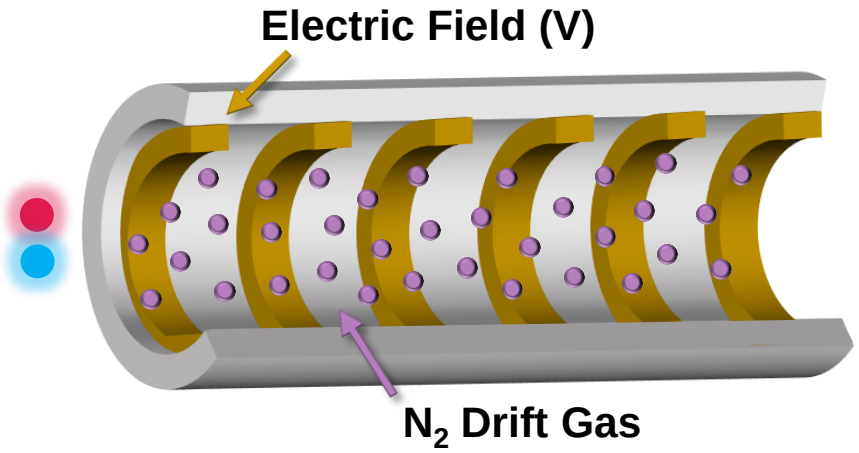
Ion Mobility Spectrometry

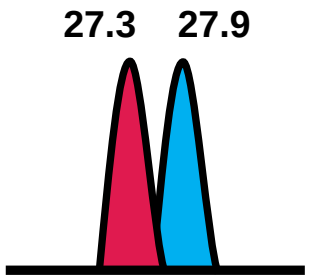
2-Methylbutyryl Carnitine
(mC5)



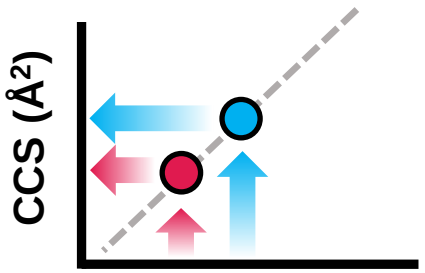
Valeryl Carnitine
(C5)



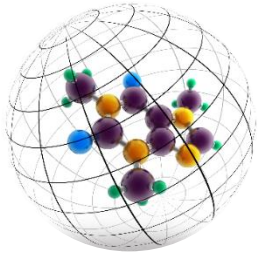


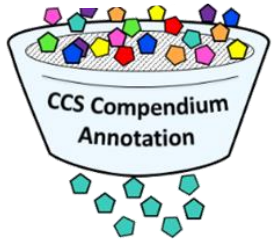


Drift Time Calibration



Collision Cross Section (CCS)
- Surface Area (Å²)

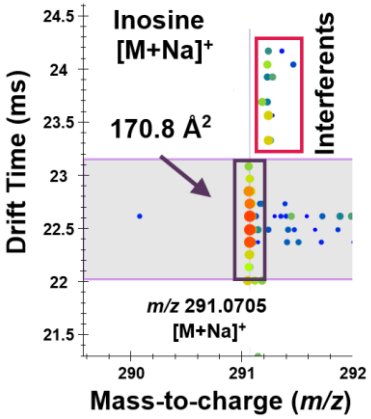




CCS Libraries

mC5 (158.6 Å²)

C5 (160.9 Å²)

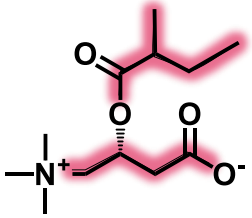


Skyline

Picache, J. A. *et. al. Chem. Sci.* **2019**, *10*, 983-993.

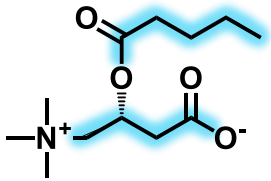
Isomer Separations

2-Methylbutyryl
(mC5)



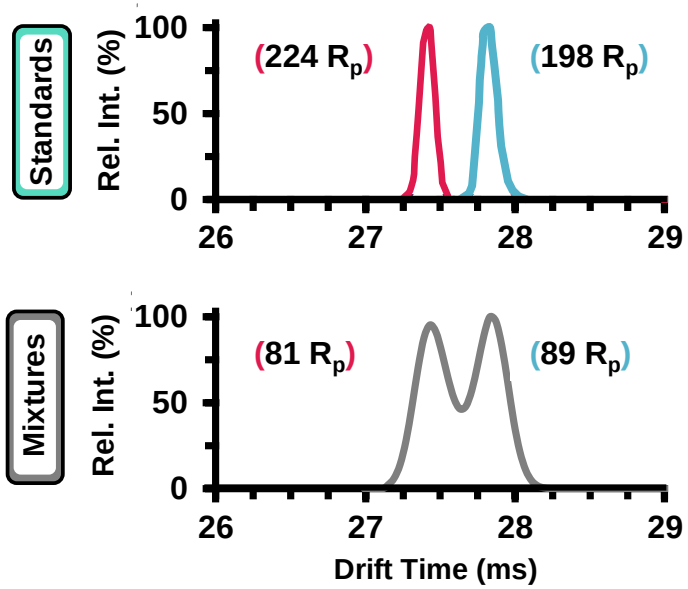
158.6 Å²

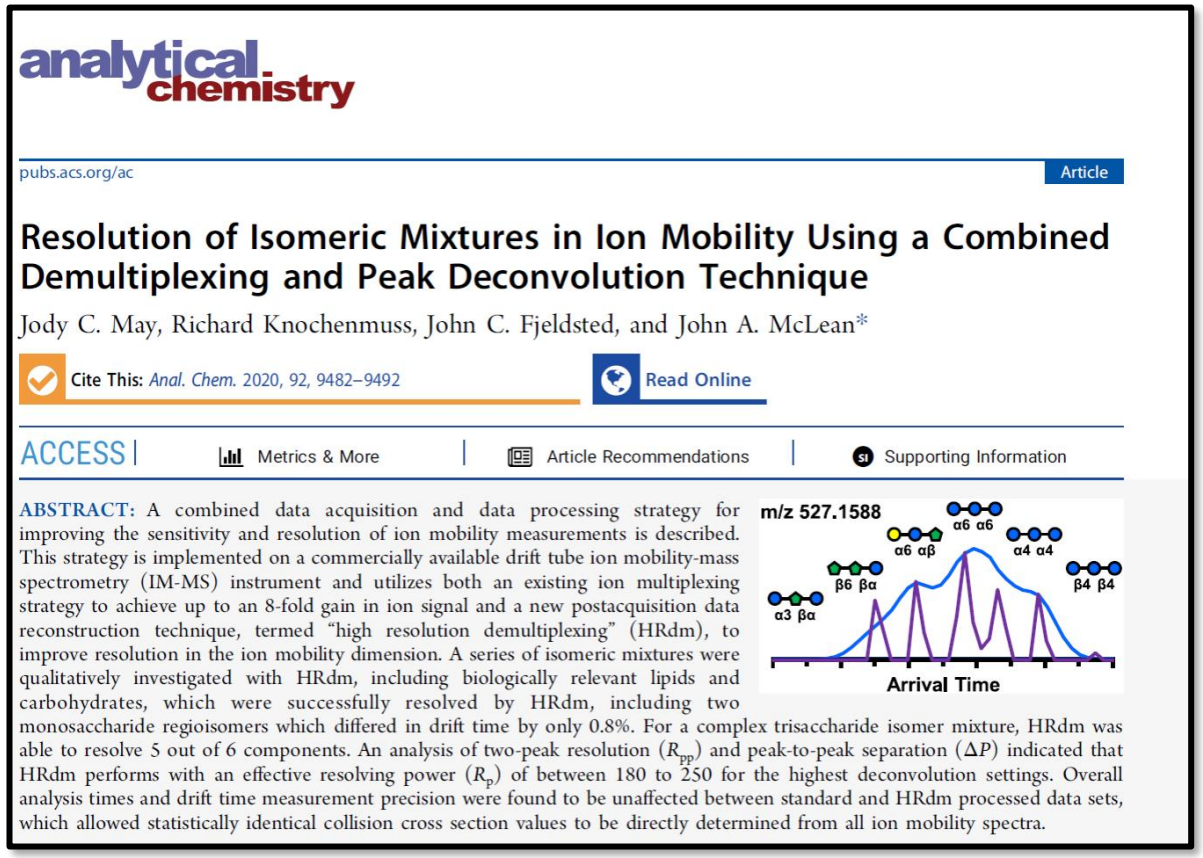
Valeryl Carnitine
(C5)



160.9 Å²

1.5% ΔCCS, 100 R_p needed
$C_{12}H_{23}NO_4$ *m/z*: 246.1705 $[M+H]^+$





analytical chemistry

pubs.acs.org/ac Article

Resolution of Isomeric Mixtures in Ion Mobility Using a Combined Demultiplexing and Peak Deconvolution Technique

Jody C. May, Richard Knochenmuss, John C. Fjeldsted, and John A. McLean*

Cite This: *Anal. Chem.* 2020, 92, 9482−9492 Read Online

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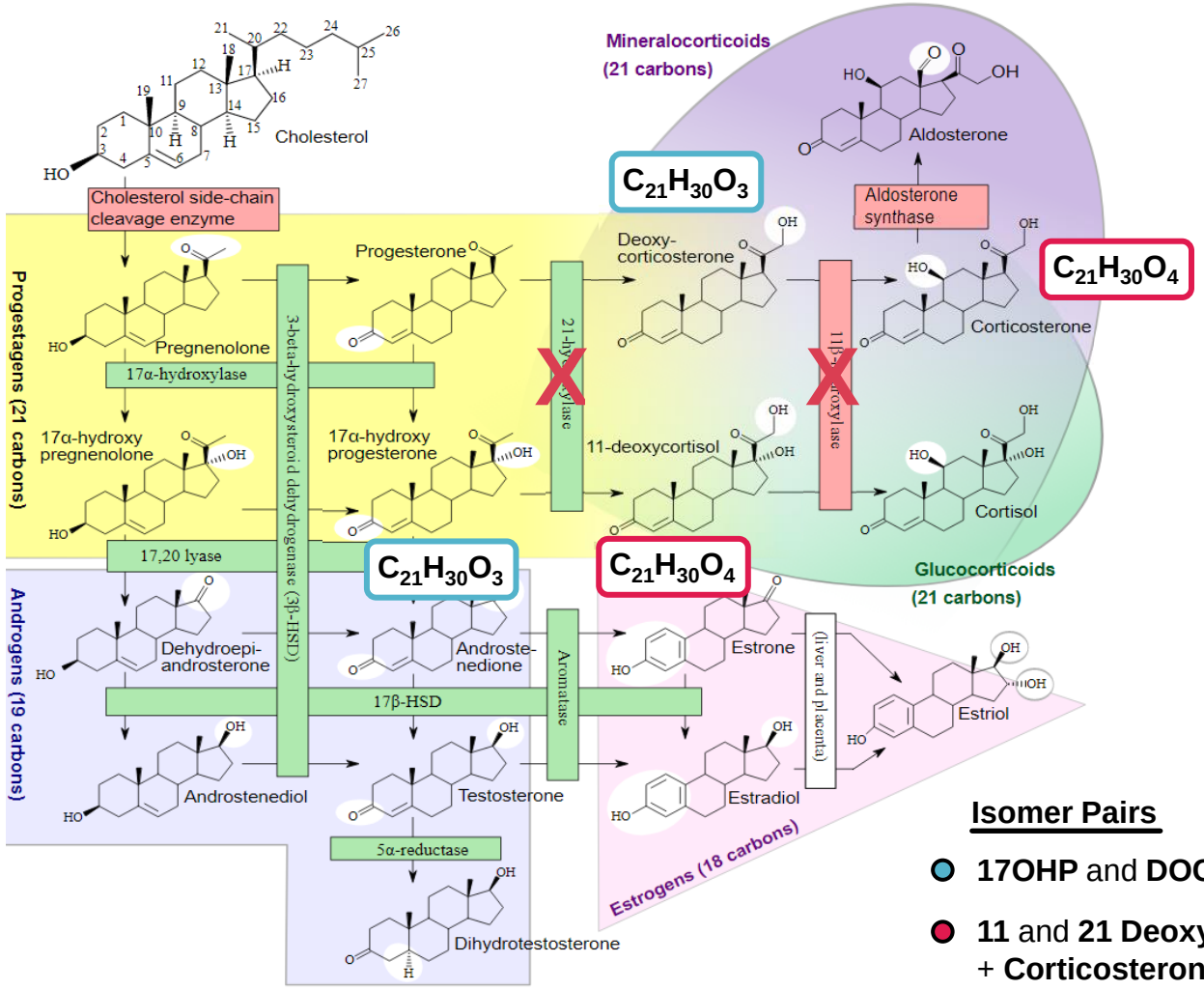
ABSTRACT: A combined data acquisition and data processing strategy for improving the sensitivity and resolution of ion mobility measurements is described. This strategy is implemented on a commercially available drift tube ion mobility-mass spectrometry (IM-MS) instrument and utilizes both an existing ion multiplexing strategy to achieve up to an 8-fold gain in ion signal and a new postacquisition data reconstruction technique, termed "high resolution demultiplexing" (HRdm), to improve resolution in the ion mobility dimension. A series of isomeric mixtures were qualitatively investigated with HRdm, including biologically relevant lipids and carbohydrates, which were successfully resolved by HRdm, including two monosaccharide regioisomers which differed in drift time by only 0.8%. For a complex trisaccharide isomer mixture, HRdm was able to resolve 5 out of 6 components. An analysis of two-peak resolution (R_{pp}) and peak-to-peak separation (ΔP) indicated that HRdm performs with an effective resolving power (R_p) of between 180 to 250 for the highest deconvolution settings. Overall analysis times and drift time measurement precision were found to be unaffected between standard and HRdm processed data sets, which allowed statistically identical collision cross section values to be directly determined from all ion mobility spectra.

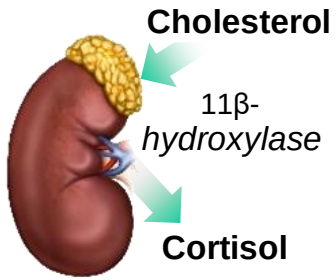
Jody May
McLean Lab – Vanderbilt

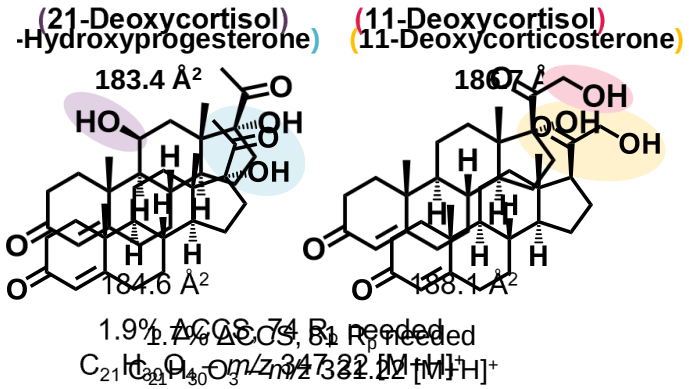
John Fjeldsted
Agilent

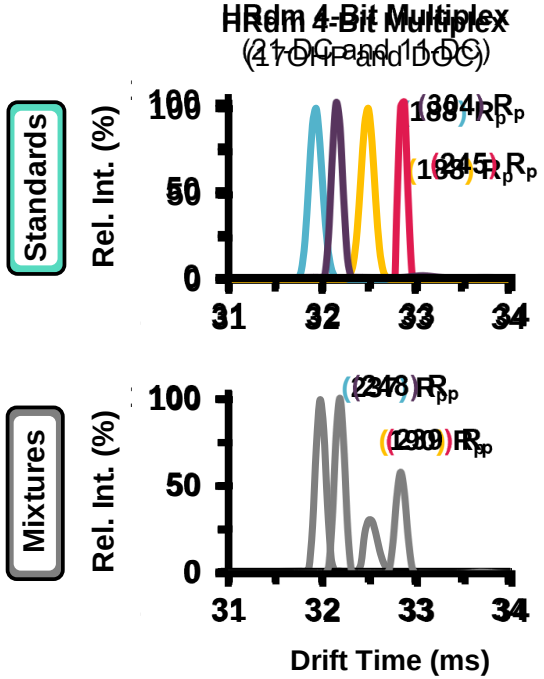
May, J. C. *et. al. Anal. Chem.* **2020**, *92*, 9482-9492.

Congenital Adrenal Hyperplasia (CAH)







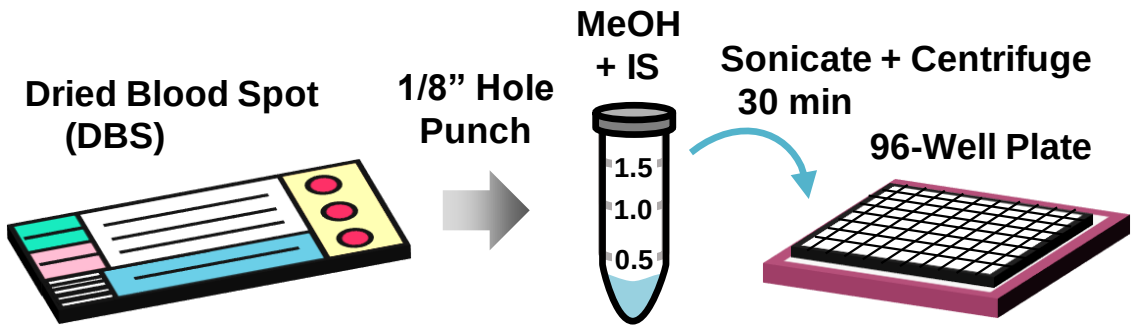


Isomer Pairs

- **17OHP** and **DOC**
- **11** and **21 Deoxycortisol + Corticosterone**

Richfield, D. and Häggström, M. "Diagram of the pathways of human steroidogenesis." *WikiJournal of Medicine.*

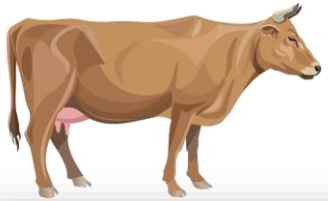
NBS Workflow

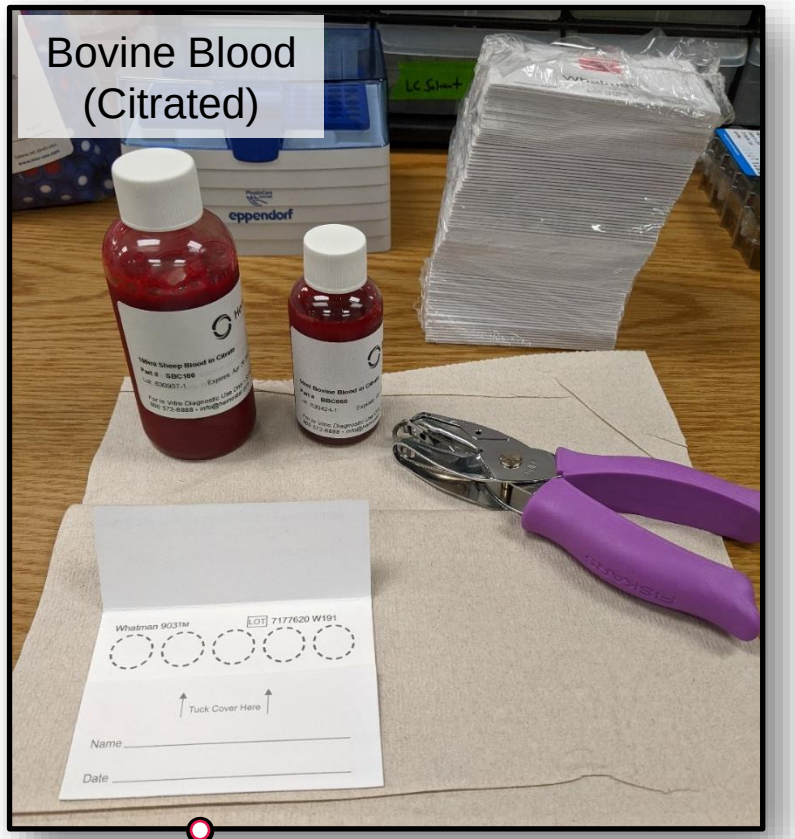


Graphitic Carbon

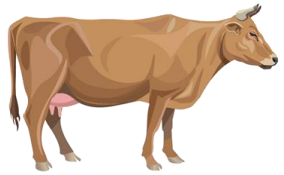
Dodds, J. N. and Baker, E. S. *Anal. Chem.* **2021**, *93*, 17094-17102.

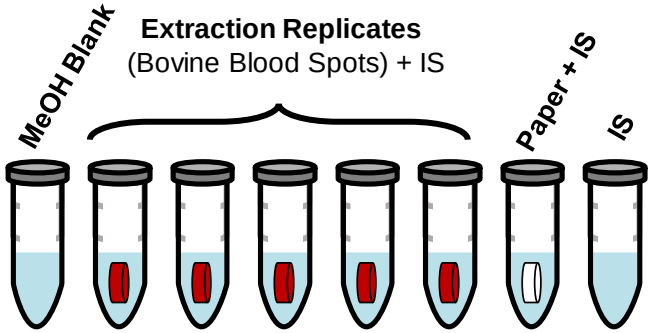
Method Reproducibility

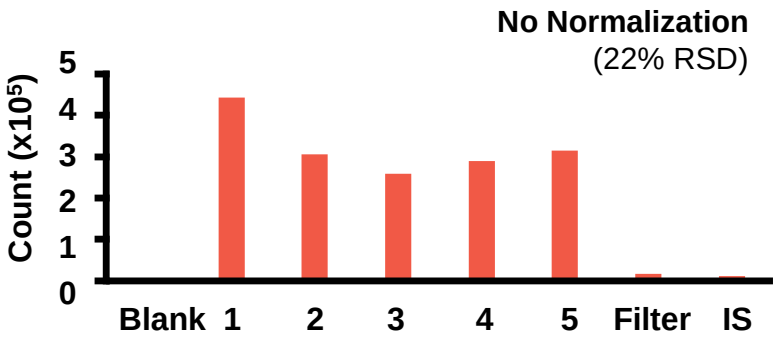


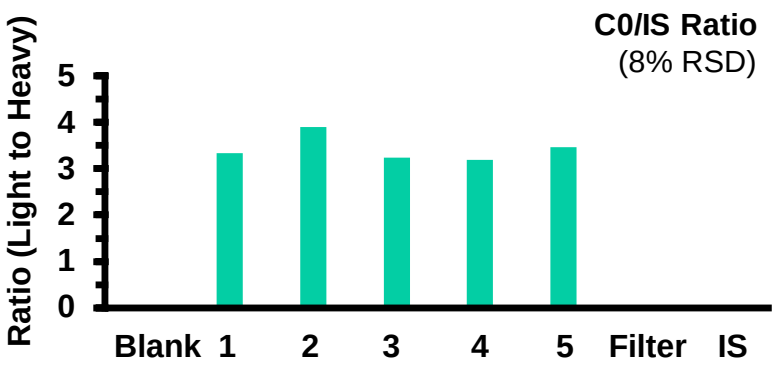


Whatman 903
Protein Saver Card

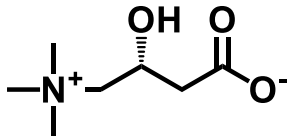


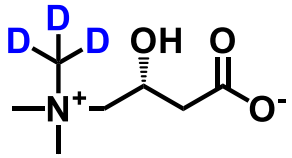






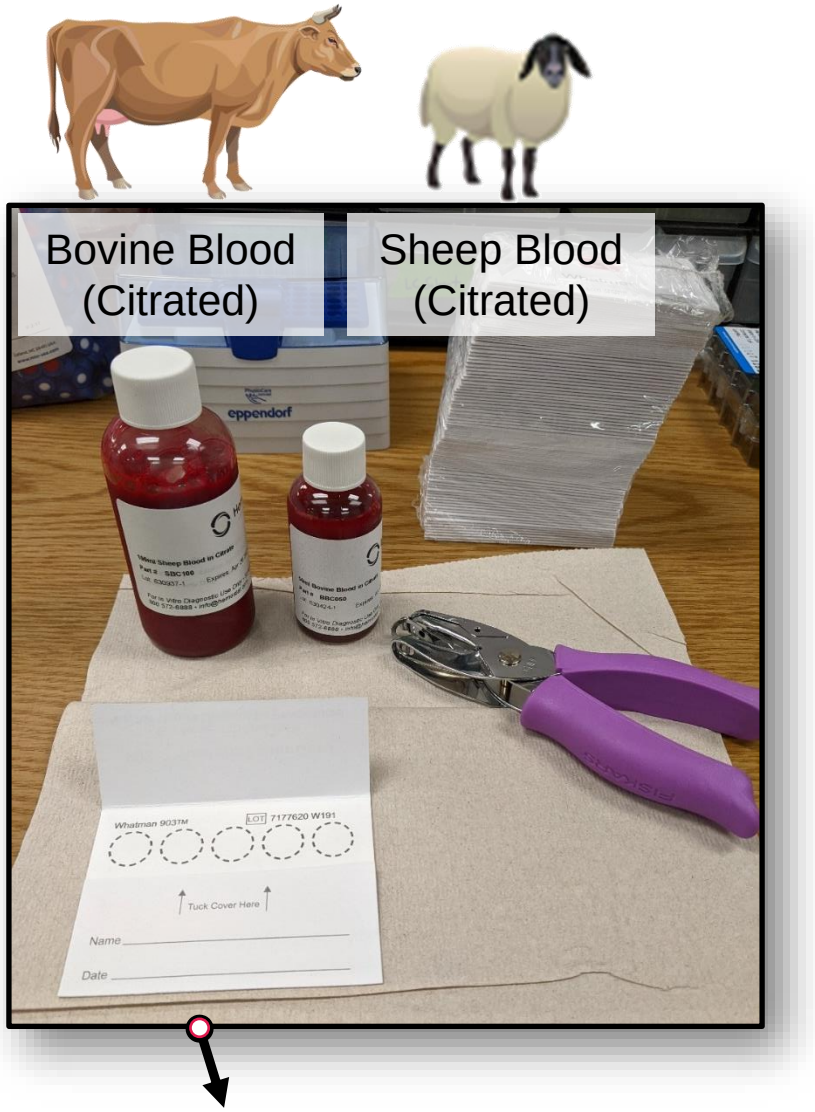
~ 20% RSD
(no normalization)



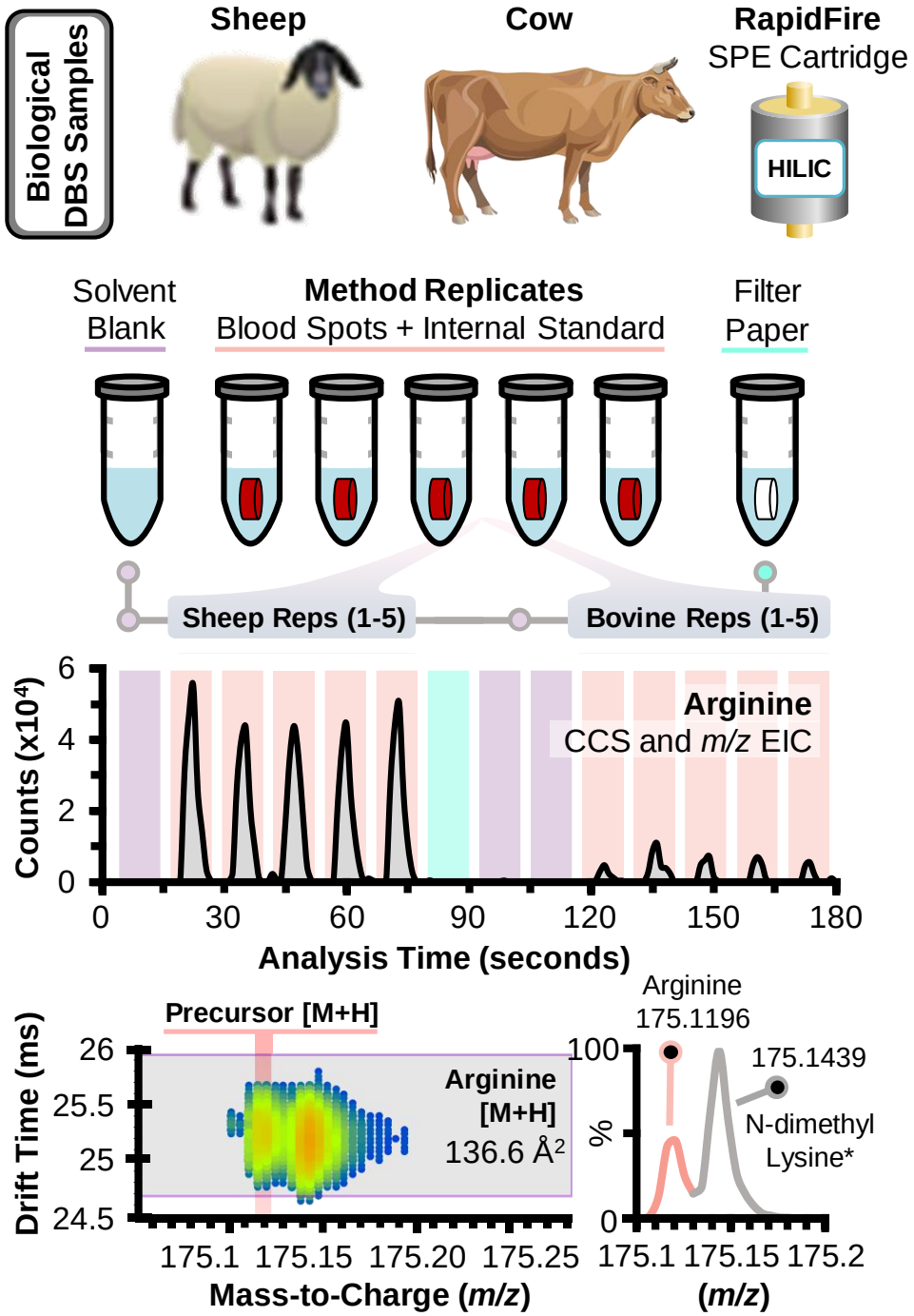


Carnitine
Carnitine-d^3

Biological Variability

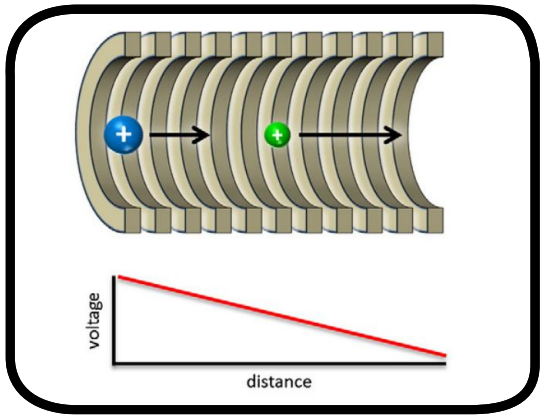


Whatman 903
Protein Saver Card



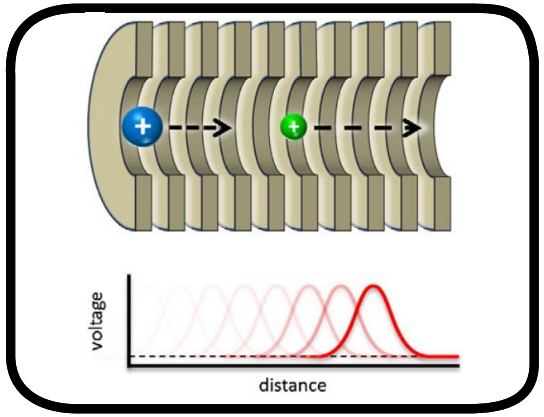
Skyline only for Drift Tube IMS?

Drift Tube (DTIMS)



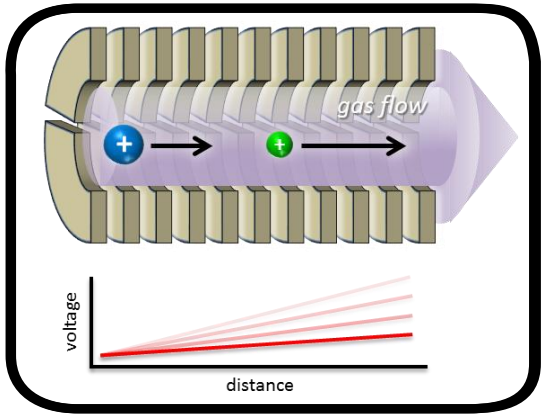
Agilent

Traveling Wave (TWIMS)



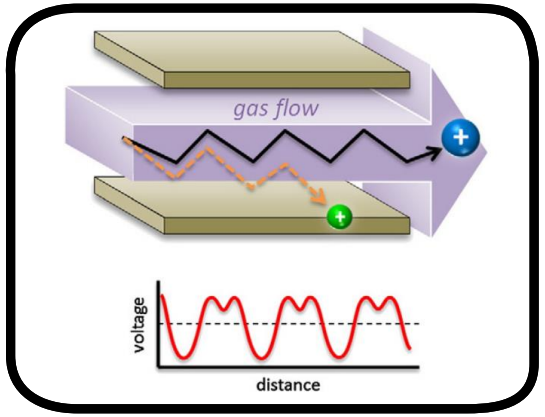
Waters, MOBILion

Trapped IM (TIMS)



Bruker

Field Asymmetric (FAIMS/DMS/DIMS)



SCIEX, Thermo (CV)

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Baker Lab Members

Principal Investigator

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- Allison Stewart
- Rebecca Beres – **WOF am**
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- Hailey Hammonds

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- MaKayla Foster
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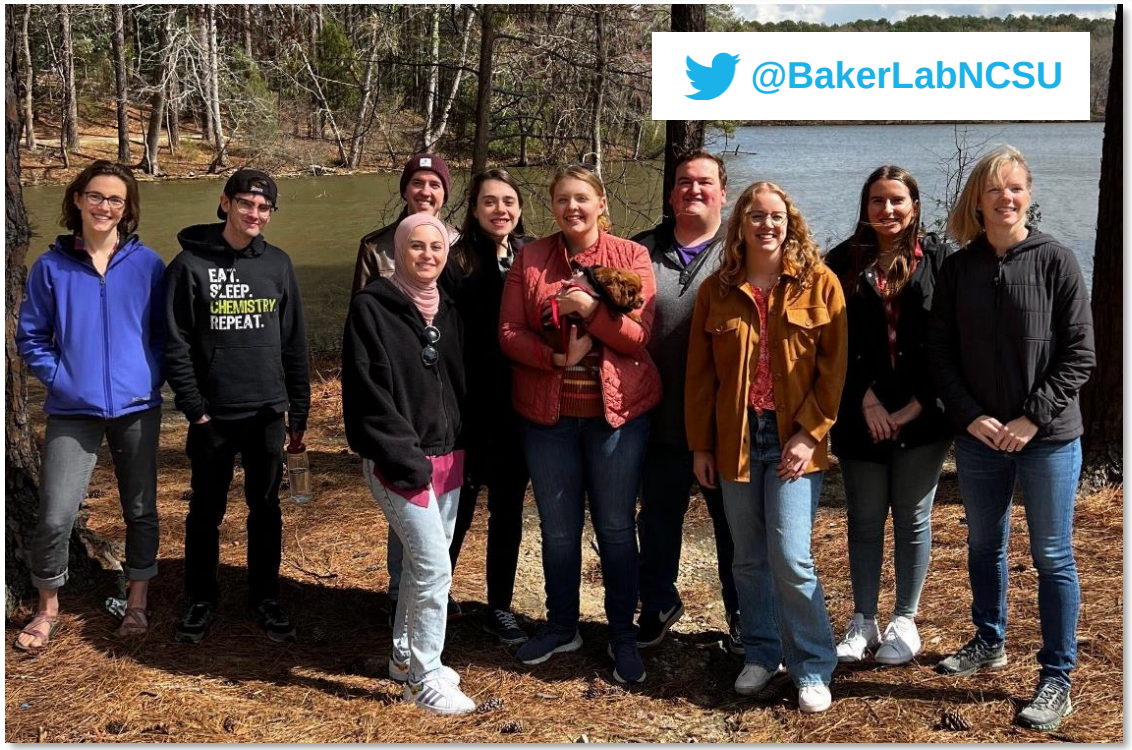
- Michael Christopher



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