Utilizing High Throughput IMS-MS Measurements to Analyze Small Molecules & Their Noncovalent Interactions with Macromolecular Complexes

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Happy Birthday Mike

Before Grad Students

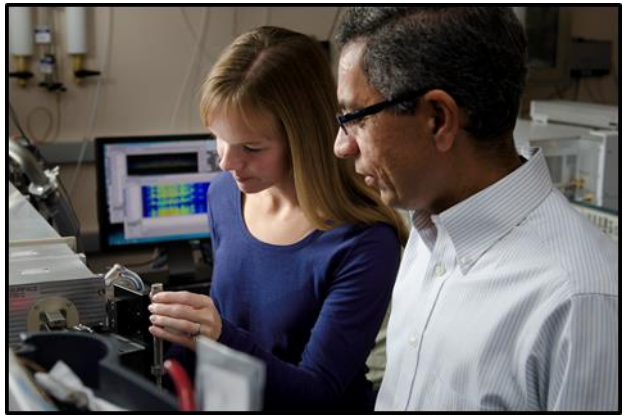
After Grad Students

Congratulations on 75 Wonderful Years Mike

Introduction

Why are we interested in using IMS-MS in small molecule studies?

1. We would like to increase throughput of measurements
2. IMS is a very fast separation technique
3. IMS-MS analyses are able to detect high feature numbers with fast LC separations or no LC separations at all
4. IMS adds complementary information to MS measurements which helps lower false discovery rates and separates isomers

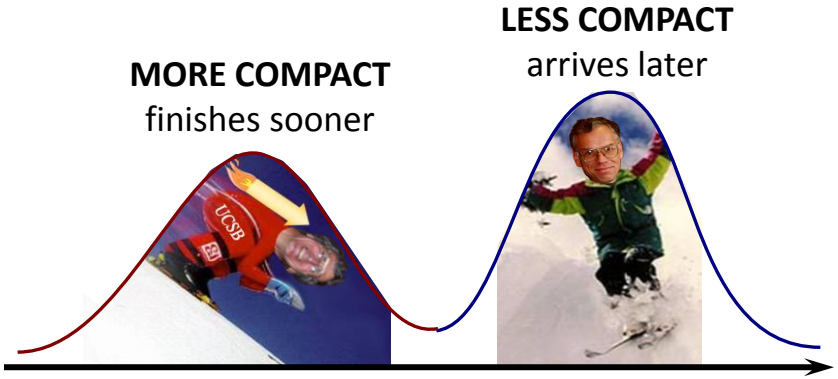


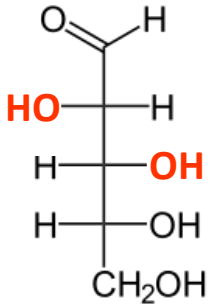
IMS Concept

No Mobility

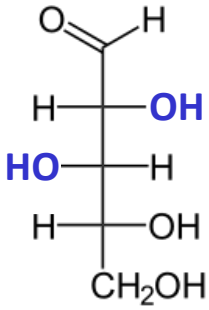
Mobility (slow)

Mobility (fast)



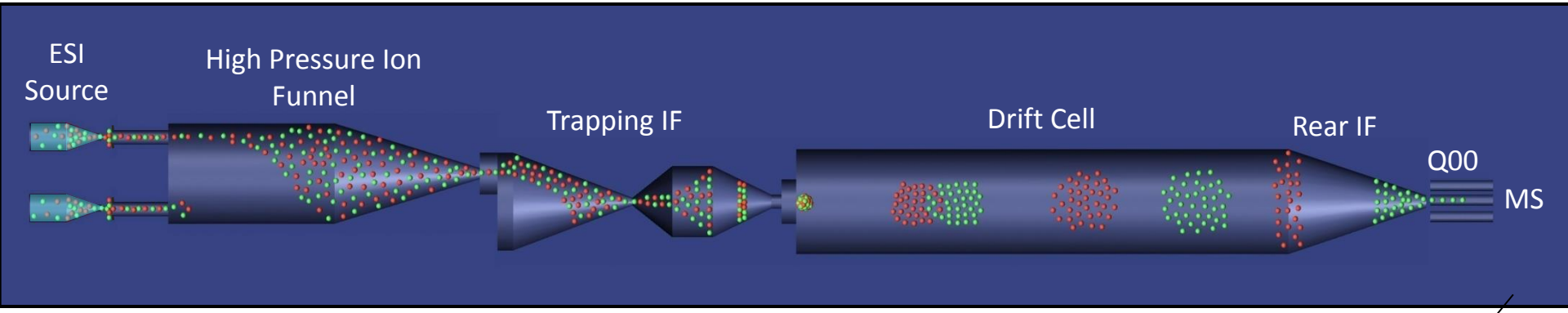


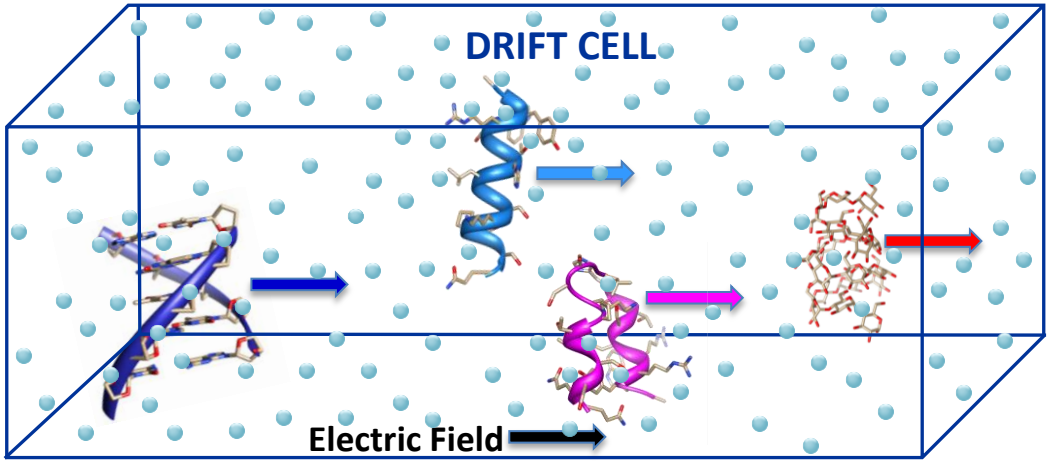
D-Arabinose

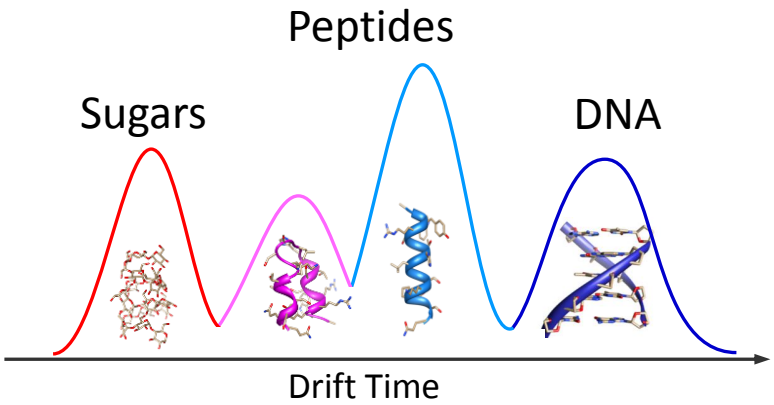


D-Xylose

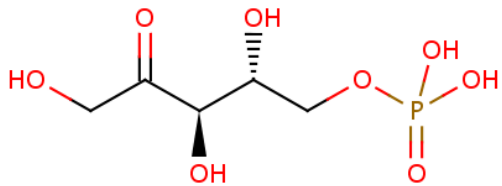
IMS-MS instrumentation



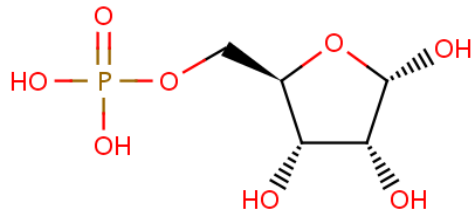




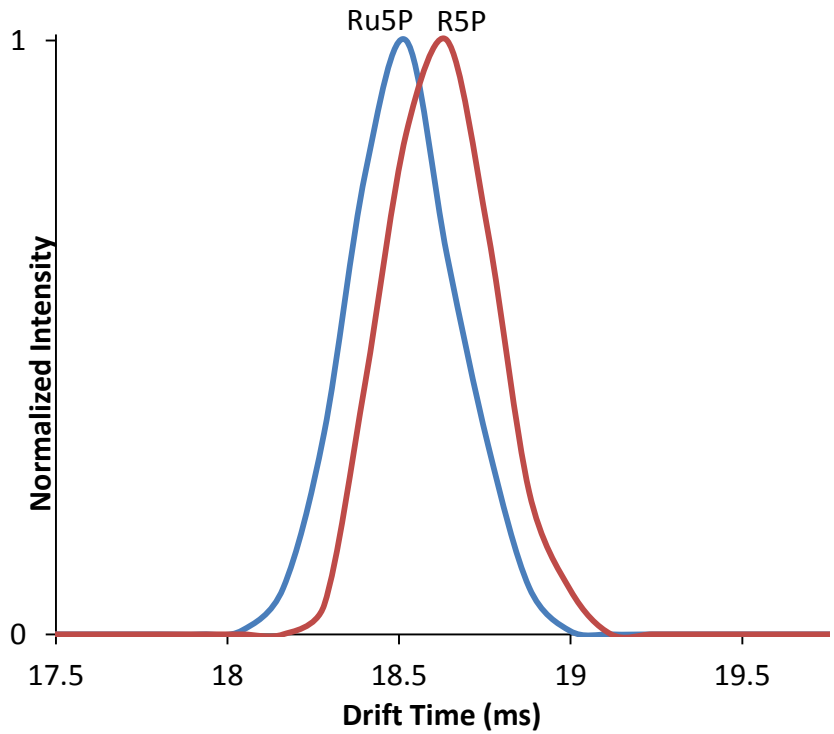
Isomer separations difficult with hydrophobic interaction liquid chromatography (HILIC)

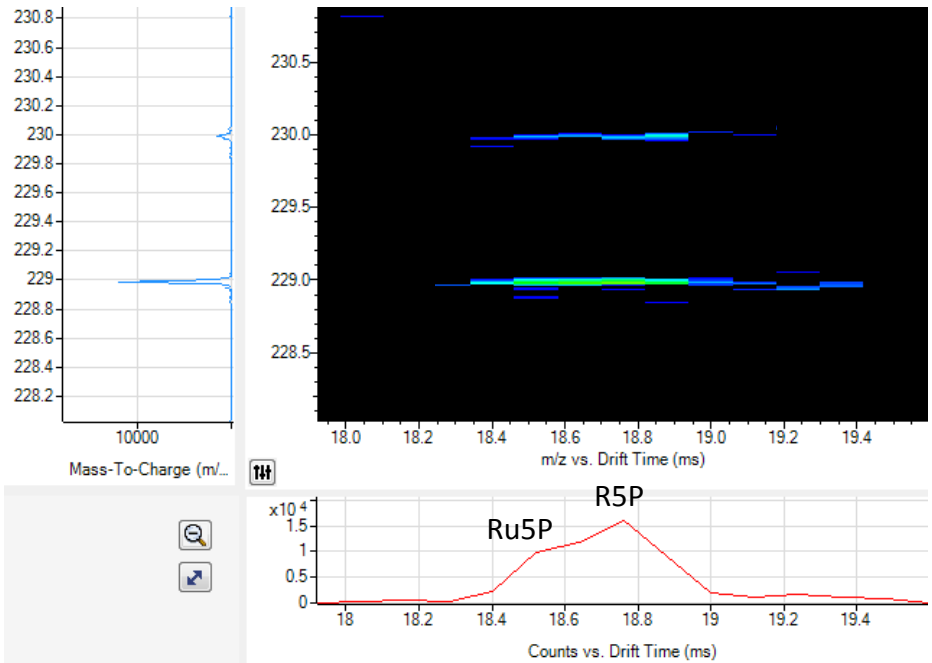


D-Ribulose 5-phosphate (Ru5P)



α-D-Ribose 5-phosphate (R5P)





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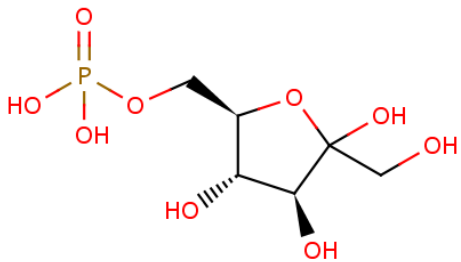
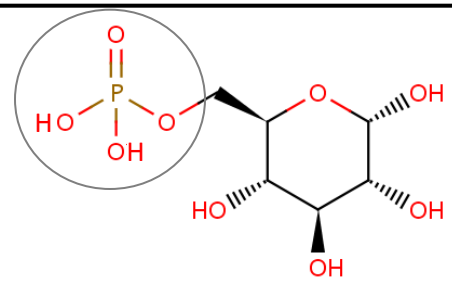
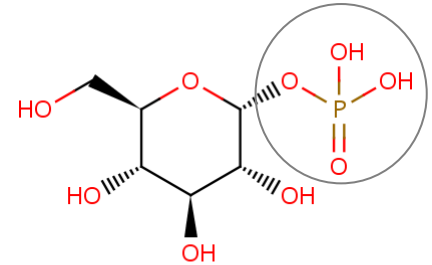


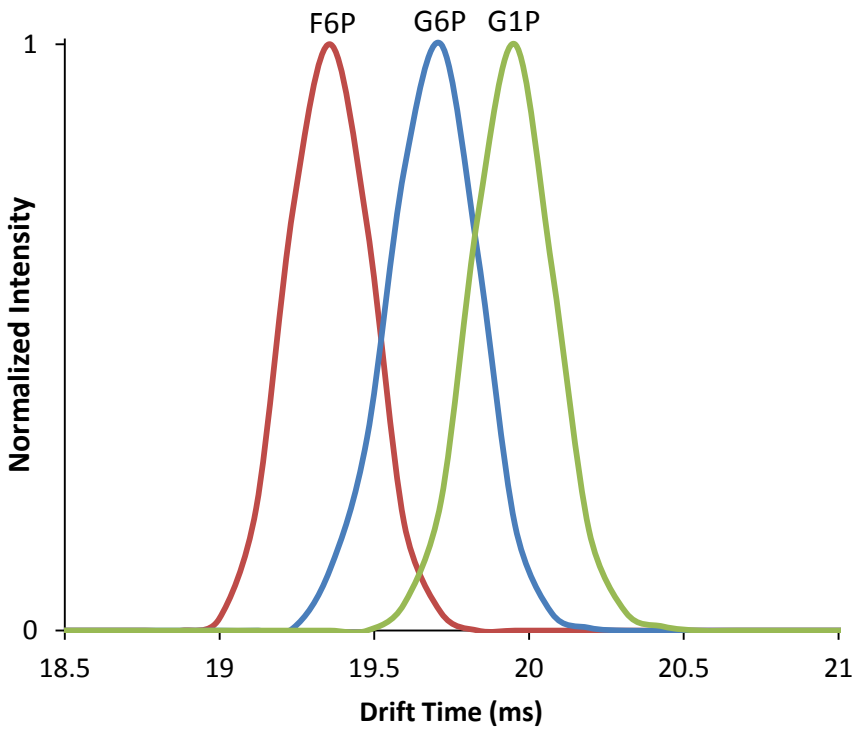
D-Fructose-6-phosphate (f6p)

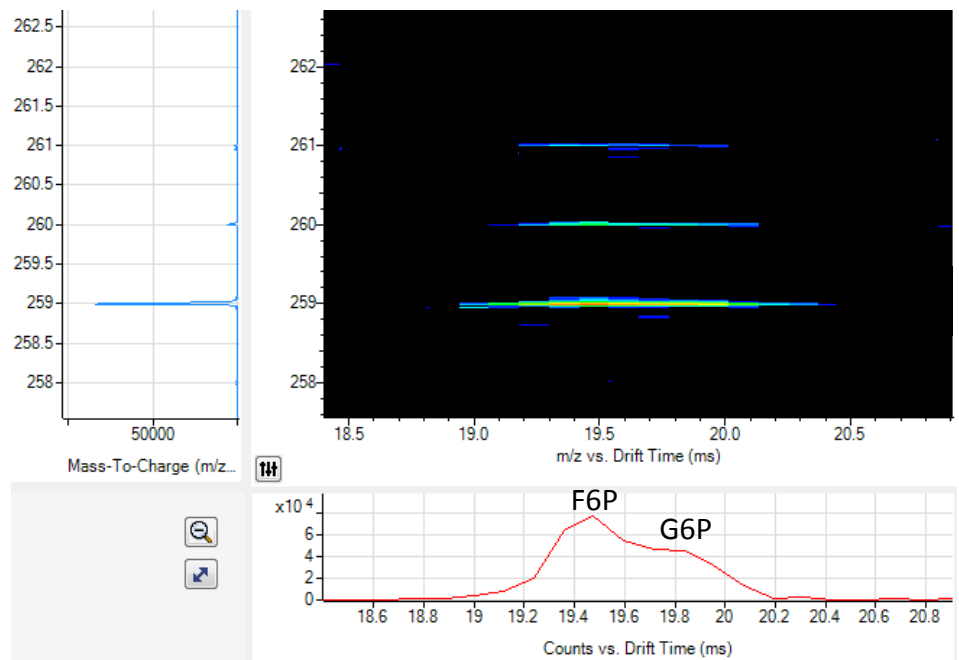


D-Glucose-6-phosphate (G6P)

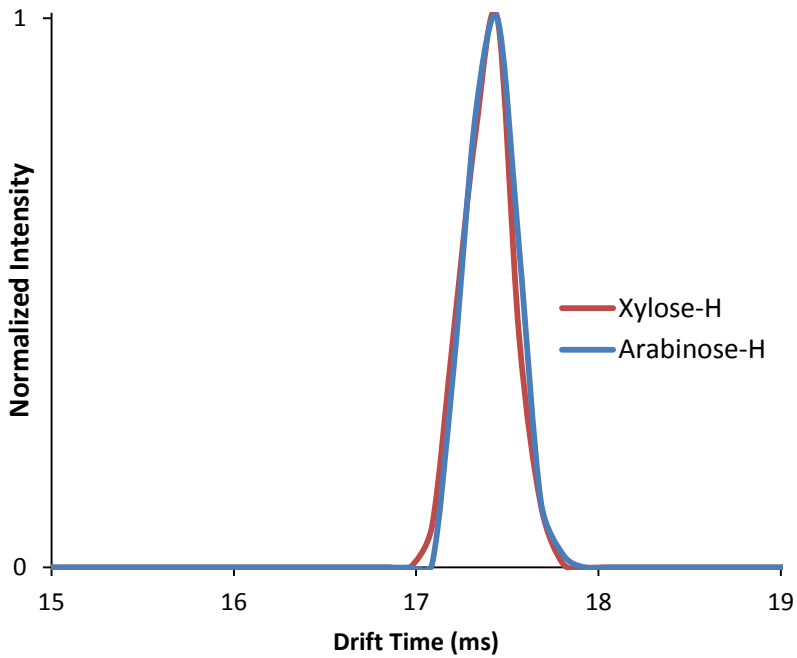


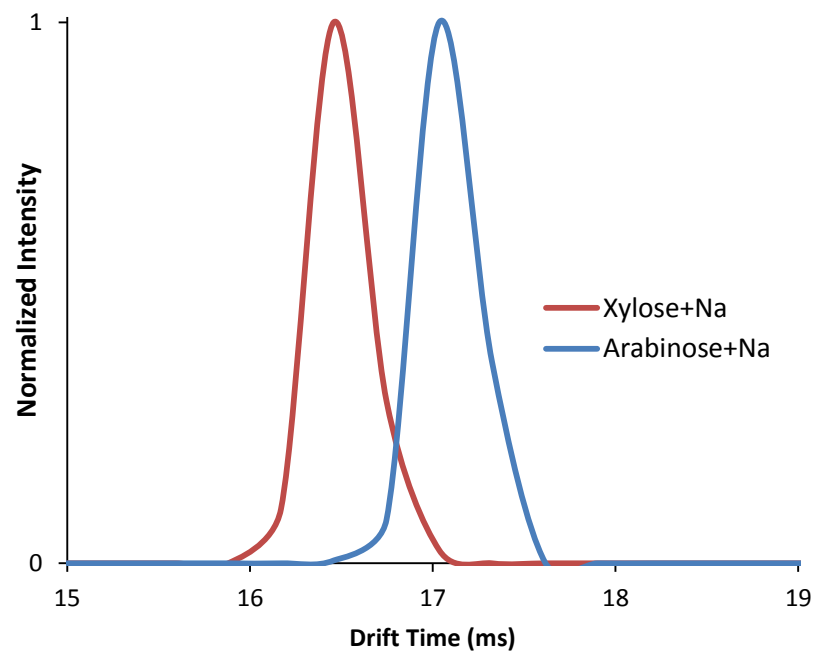
α-D-Glucose-1-phosphate (G1P)

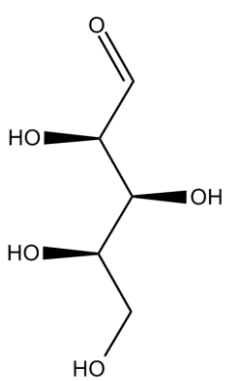




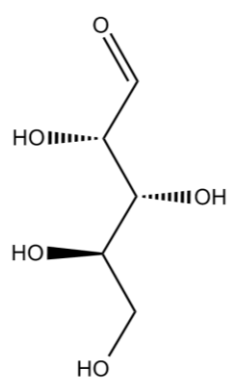
(Sugar+Na)$^+$ isomer separations with IMS





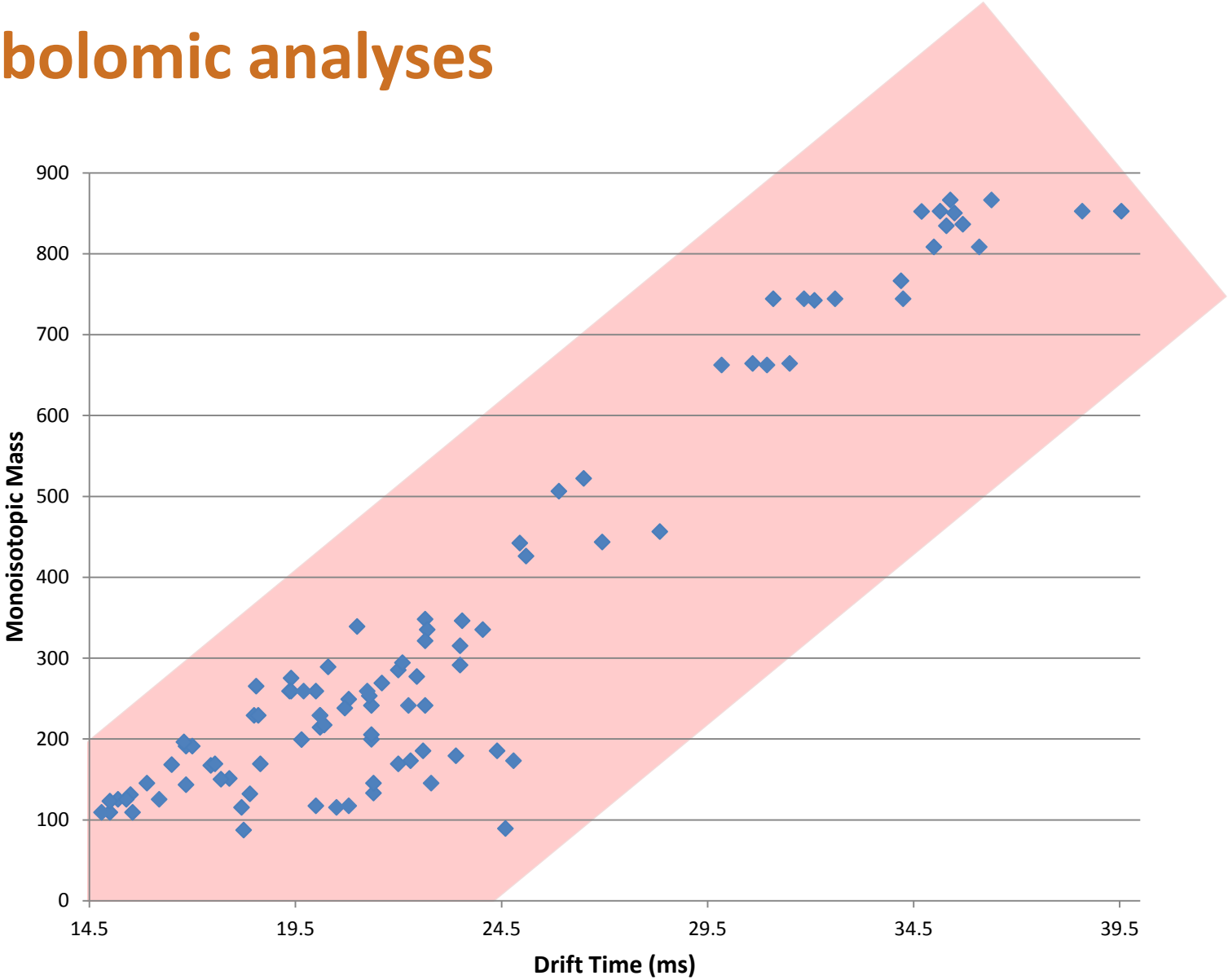


Xylose



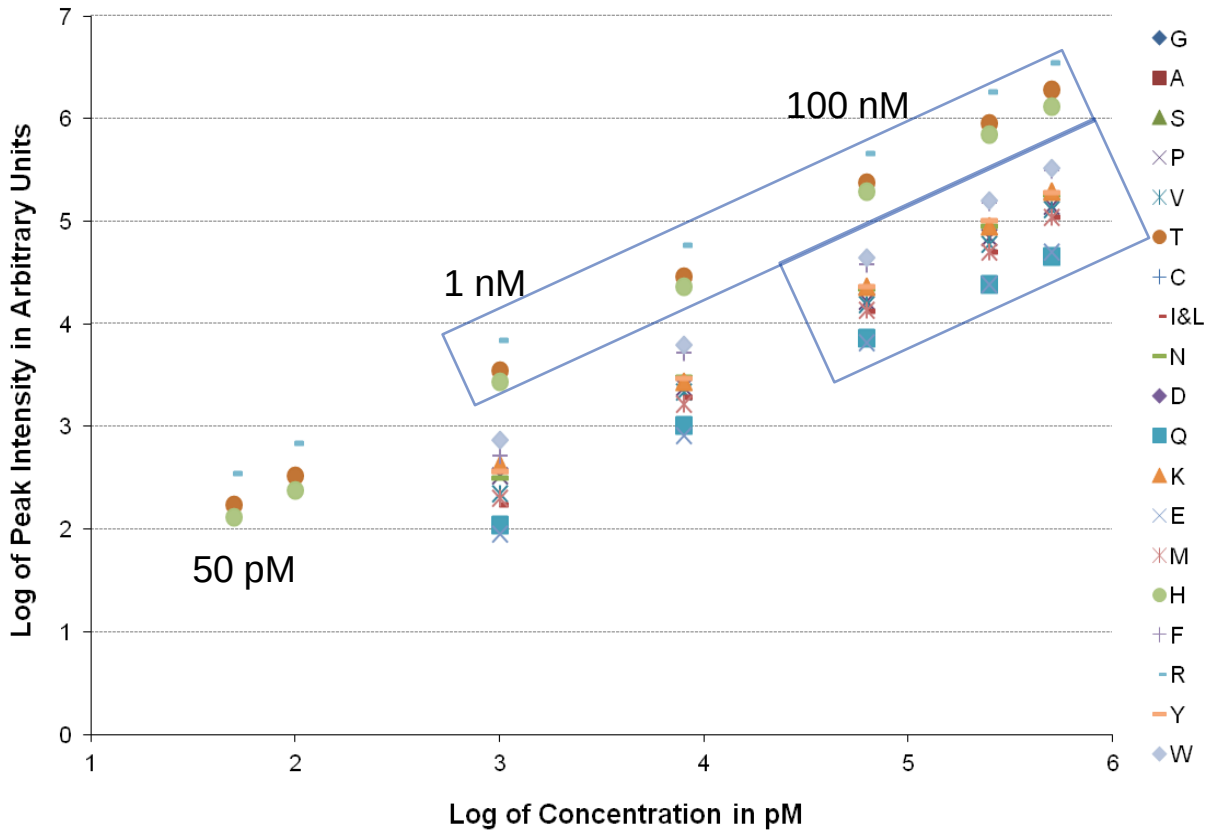
Arabinose

Metabolomic analyses



Analyzed 120 small metabolites and see orthogonality with mass

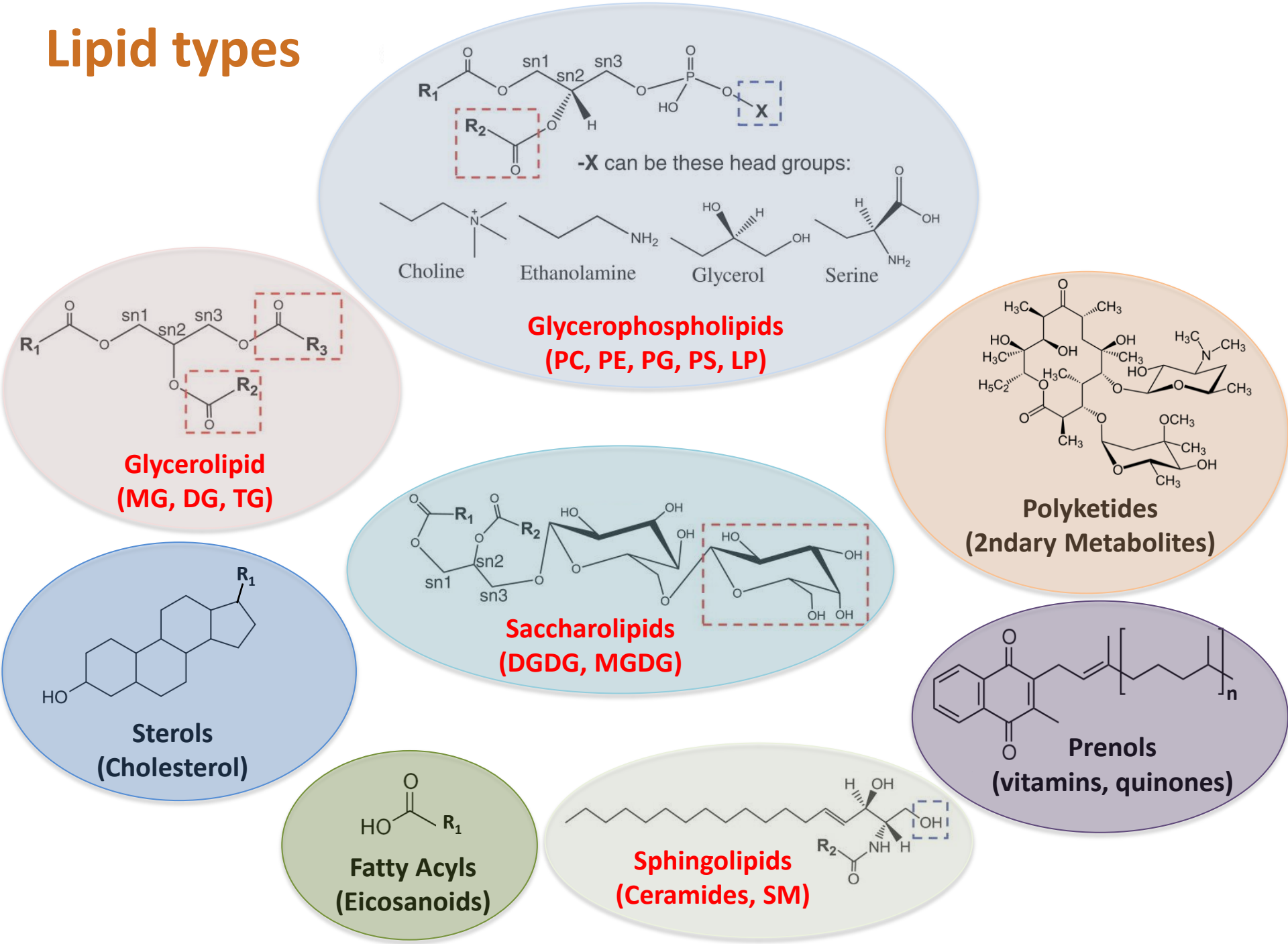
(Amino acid + H)$^+$ direct infusion concentration study



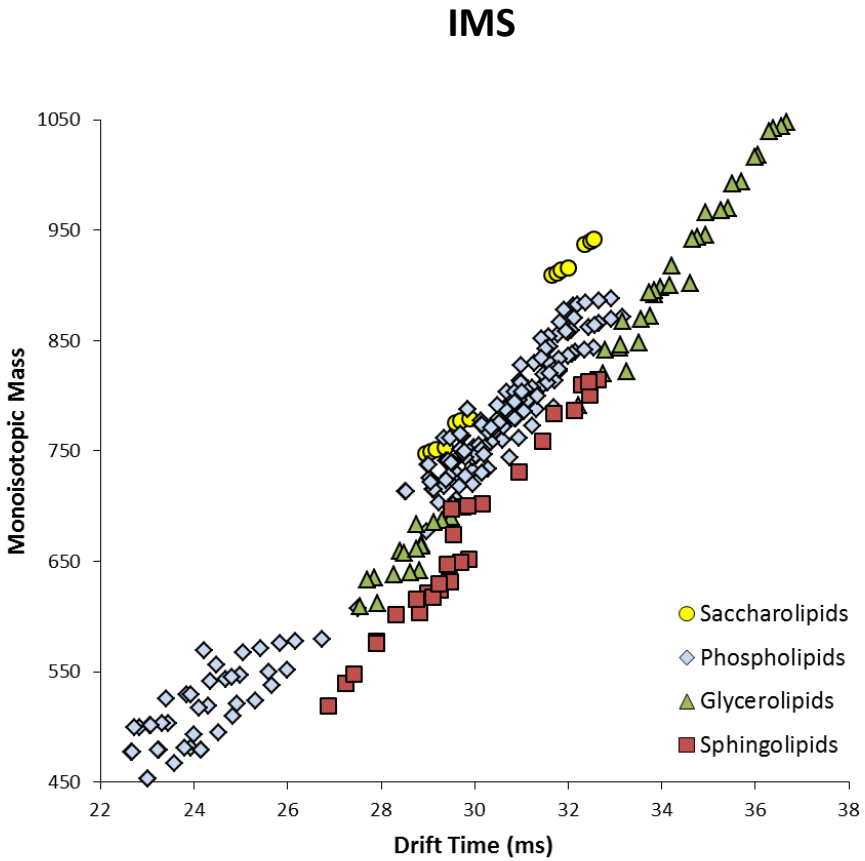
Linear concentration response

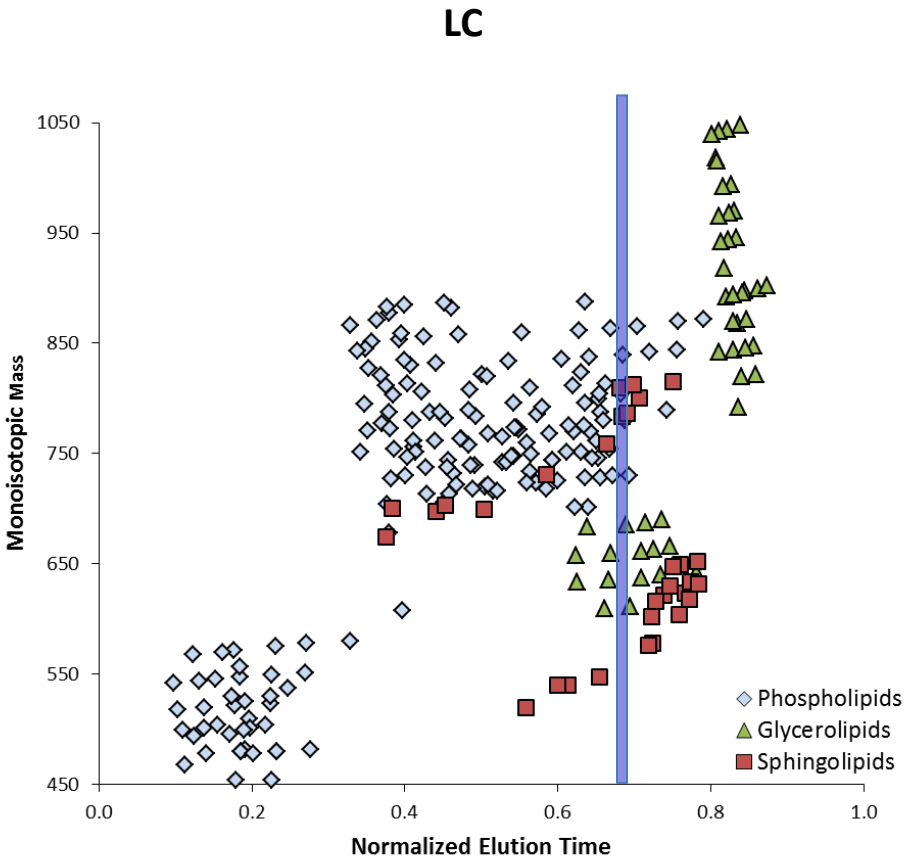
QTOF could only detect concentrations in the squares (~20x higher than IMS-QTOF MS).

Lipid types


sn1
sn2
sn3
R1
R2
X
-X can be these head groups:
Choline
Ethanolamine
Glycerol
Serine
Glycerophospholipids
(PC, PE, PG, PS, LP)
R3
Glycerolipid
(MG, DG, TG)
Polyketides
(2ndary Metabolites)
Saccharolipids
(DGDG, MGDG)
Sterols
(Cholesterol)
Prenols
(vitamins, quinones)
Fatty Acyls
(Eicosanoids)
Sphingolipids
(Ceramides, SM)

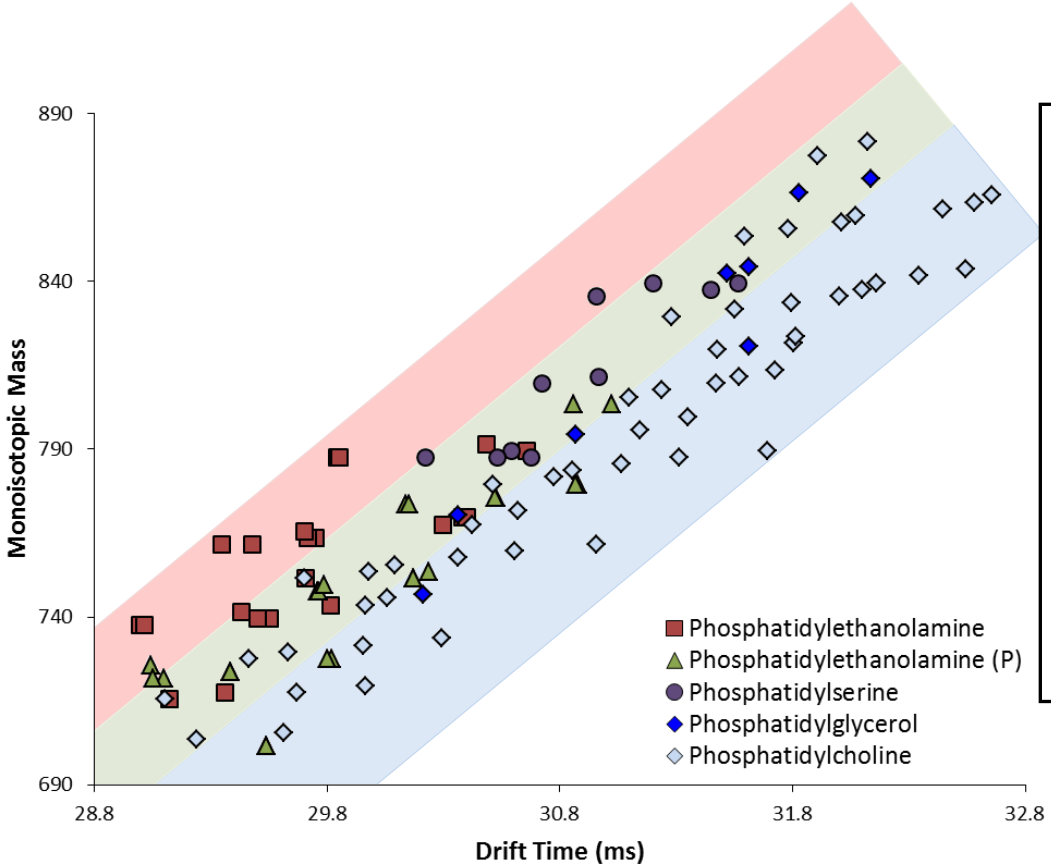
Lipid type analysis with IMS and LC

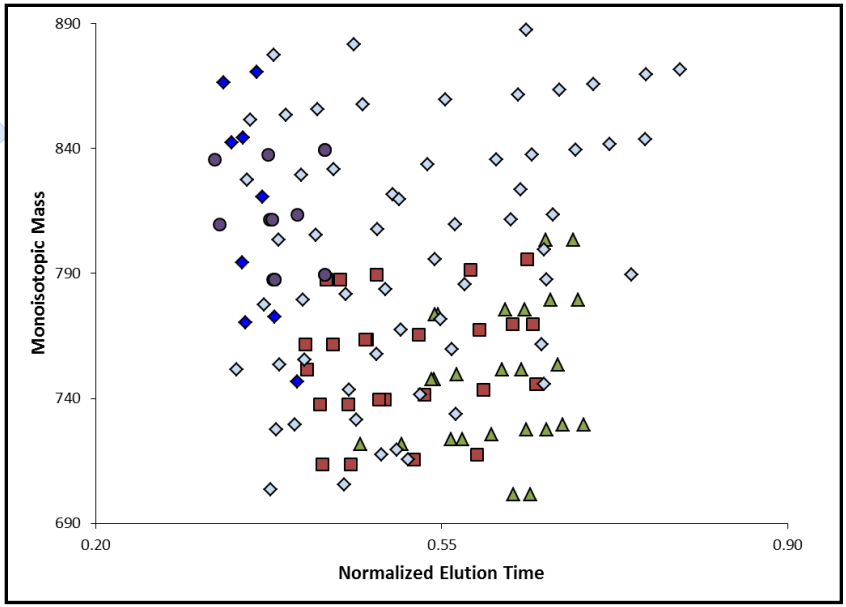




LC and IMS add complementary information for lipid identification

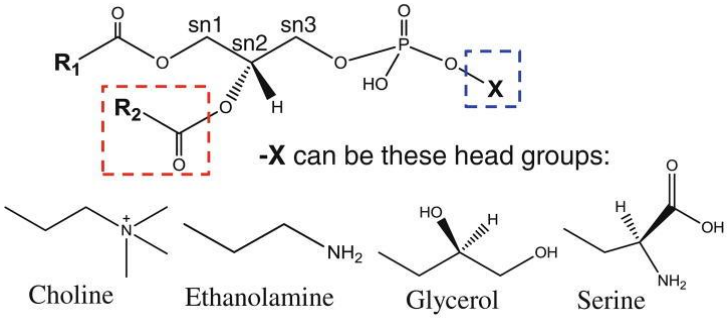
Phospholipid separation with IMS



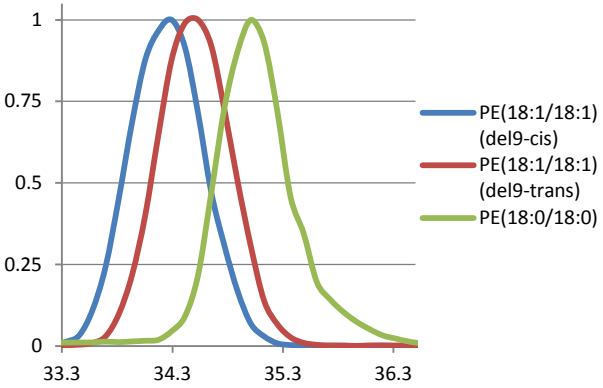


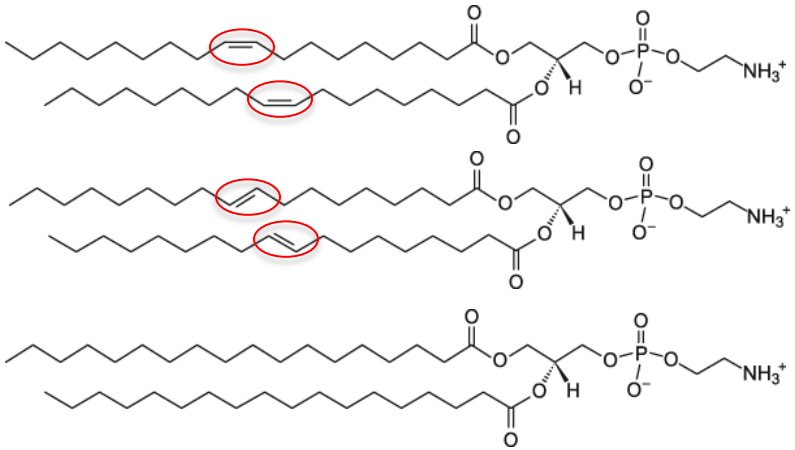
Compactness

PE < PE(P) = PS = PG < PC

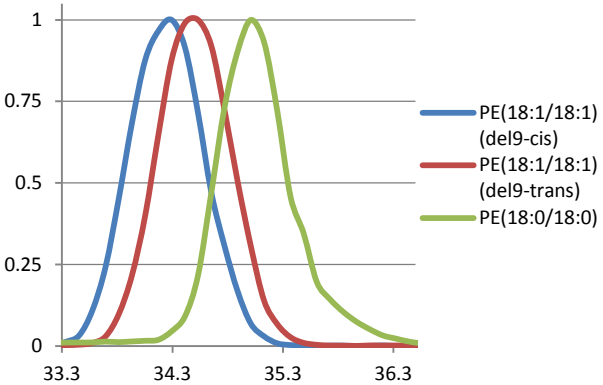


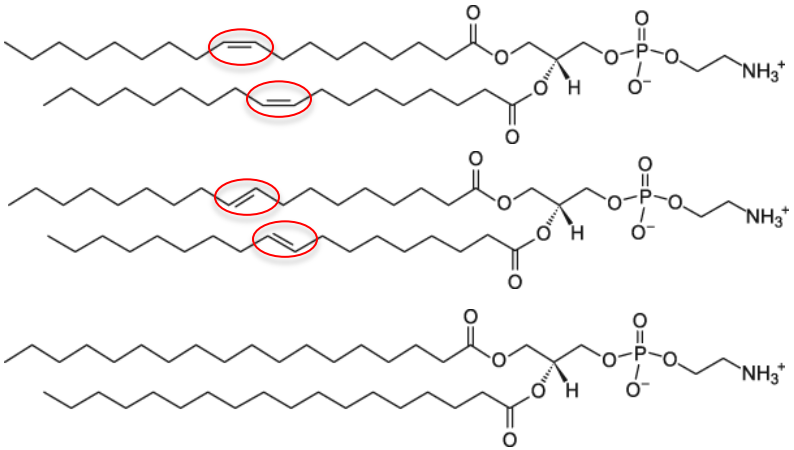
Cis/Trans double bond analysis

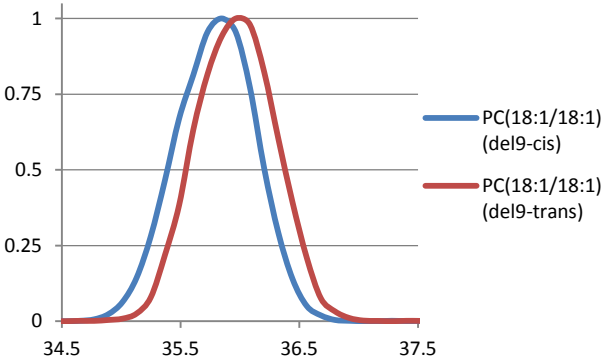


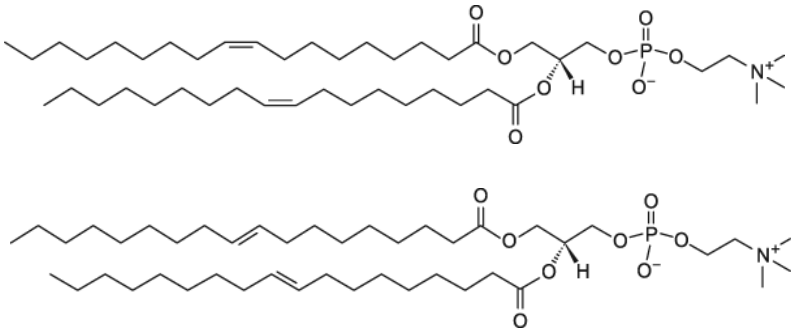


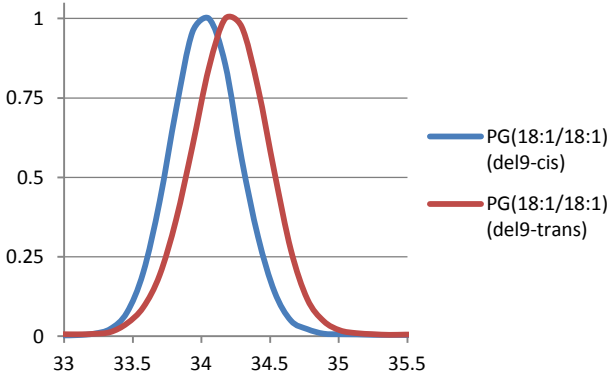
Cis/Trans double bond analysis

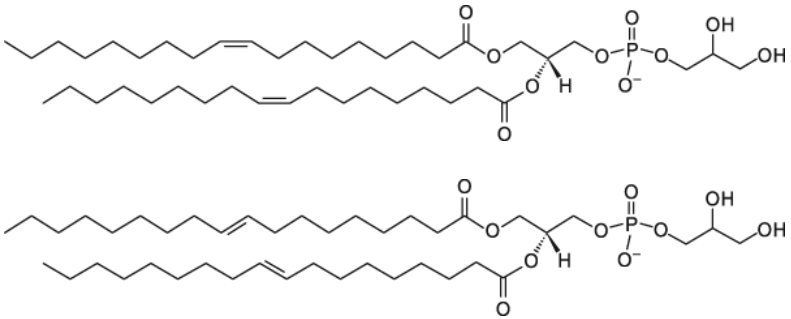




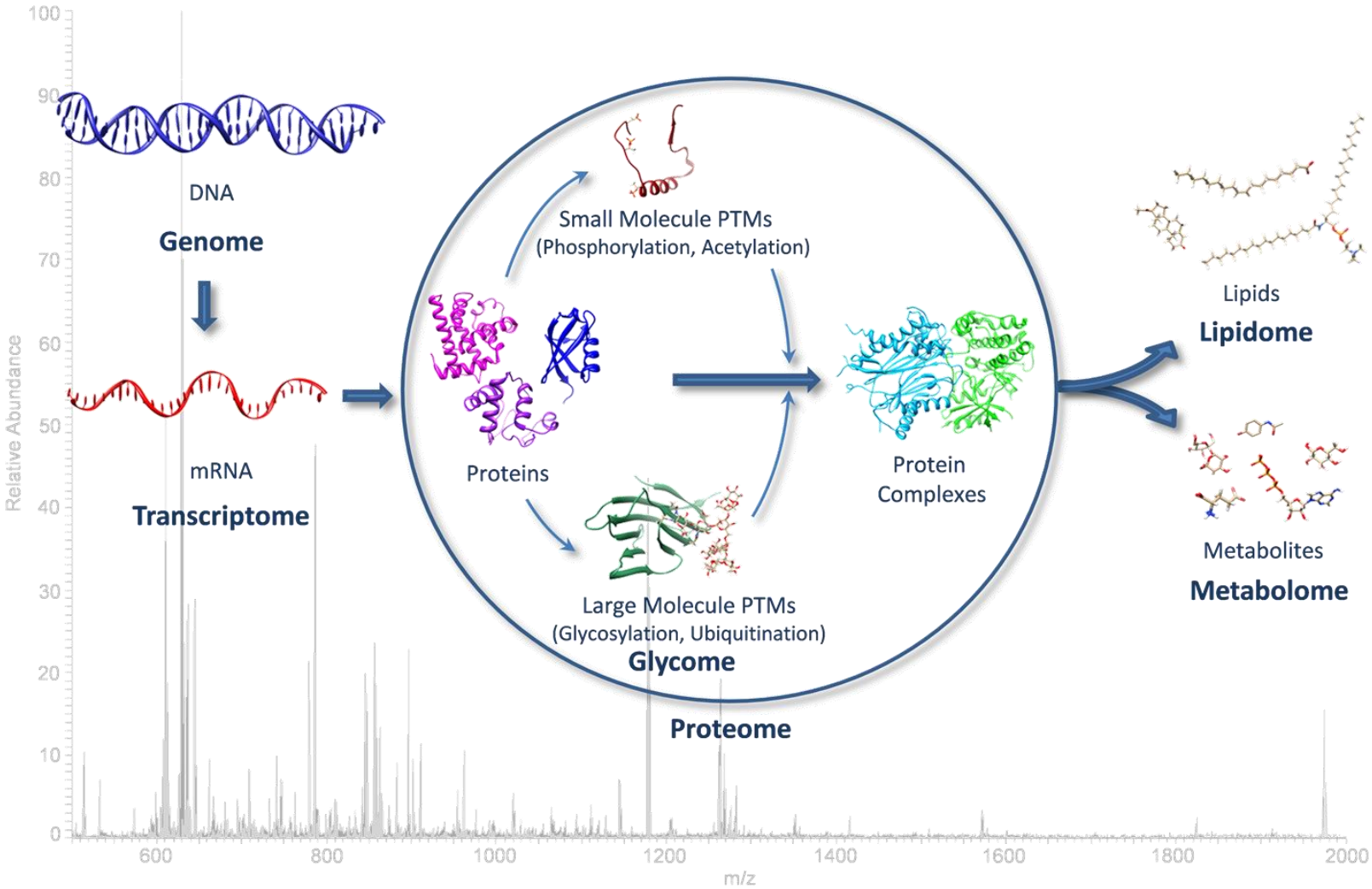






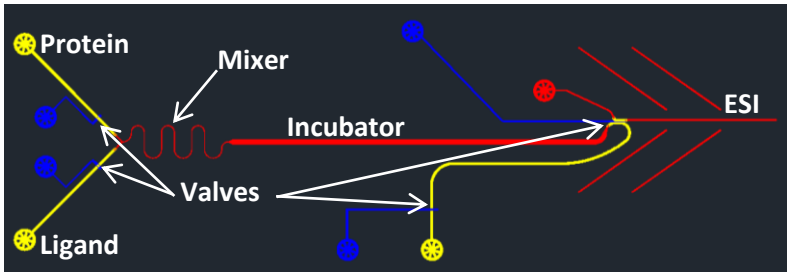


Lipidomics and Metabolomics

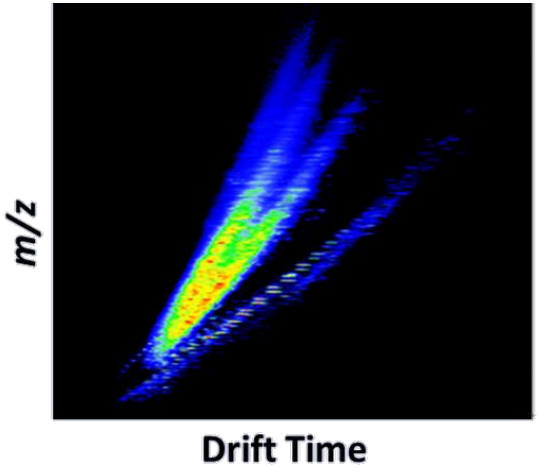


Protein/Ligand Binding Kinetics Studies

Microfluidic Chip



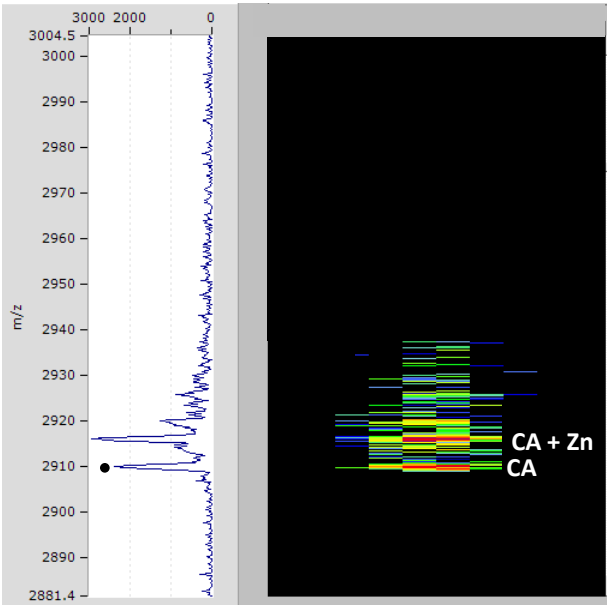
IMS-MS



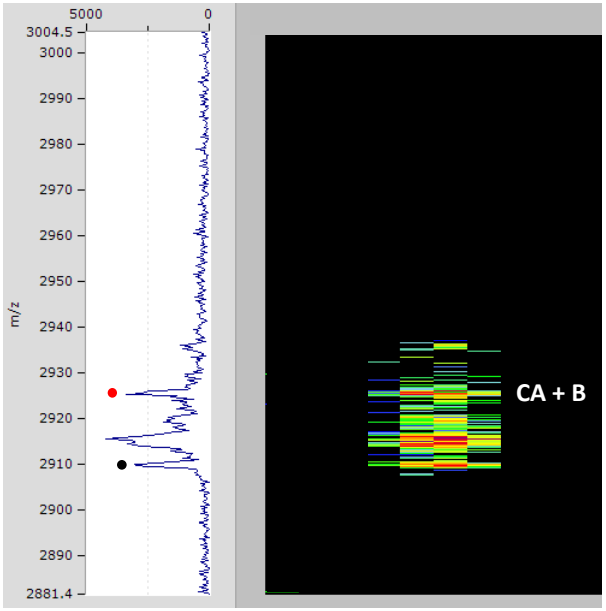
$$\frac{(P.L)}{(P)} = \frac{1}{2}\left(-1 - \frac{[P]_0 + [L]_0}{K_D} + \sqrt{4\frac{[L]_0}{K_D} + \left(\frac{[L]_0 - [P]_0}{K_D} - 1\right)^2}\right)$$

M. C. Jecklin et al. *J. Mol Recognit*. 2009; **22**: 319-329.

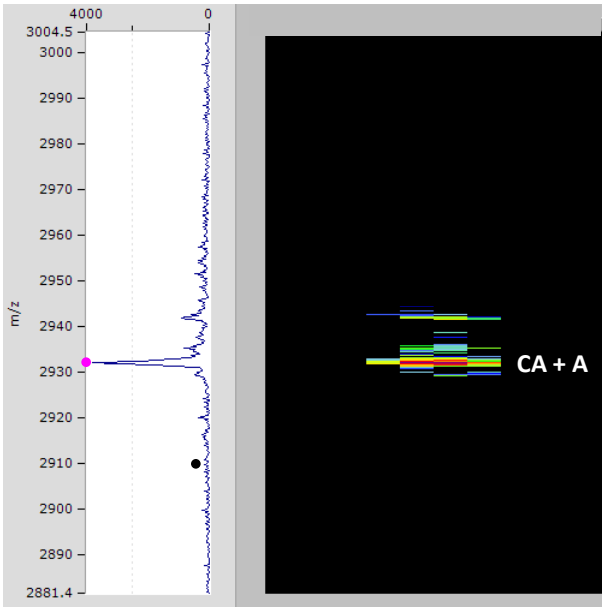
Protein complexes



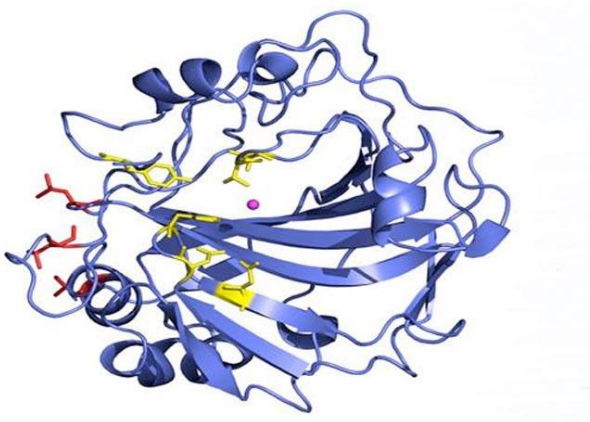
Carbonic Anhydrase

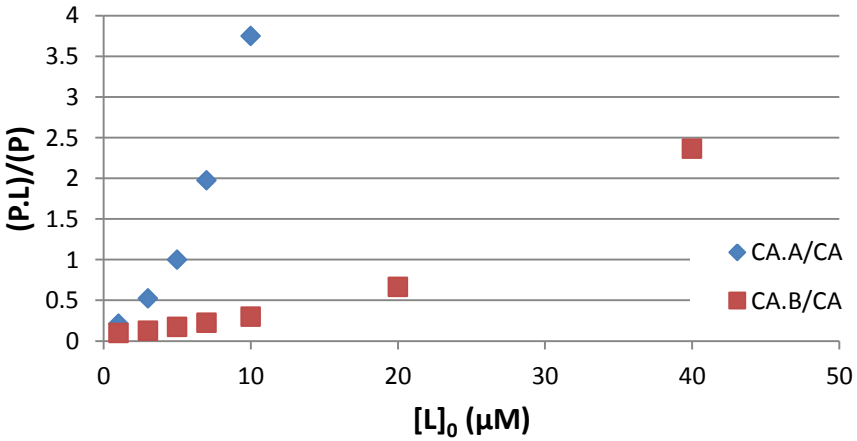


CA + Benzenesulfonamide
K_D in low µM

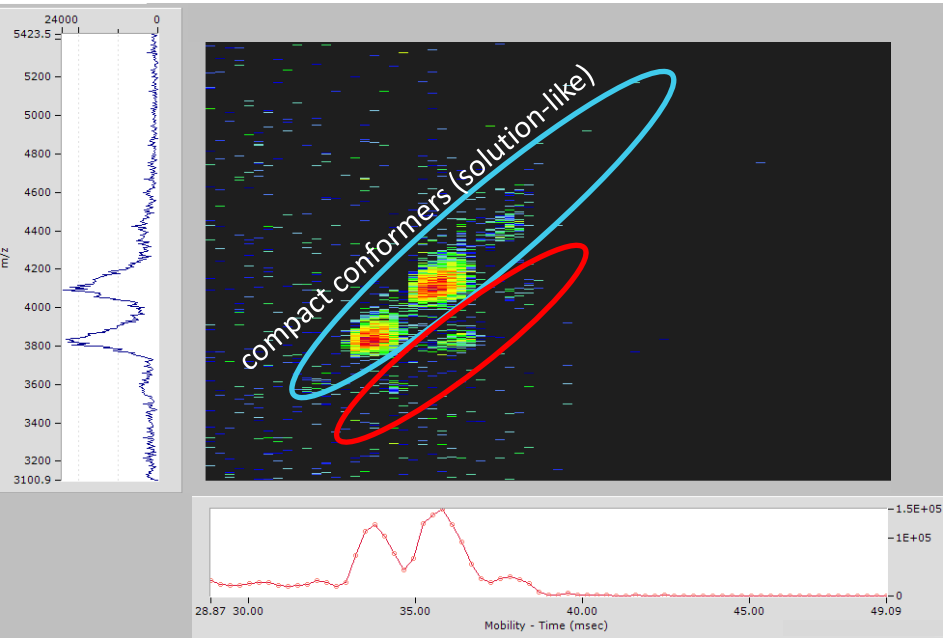


CA + Acetazolamide
K_D in low nM



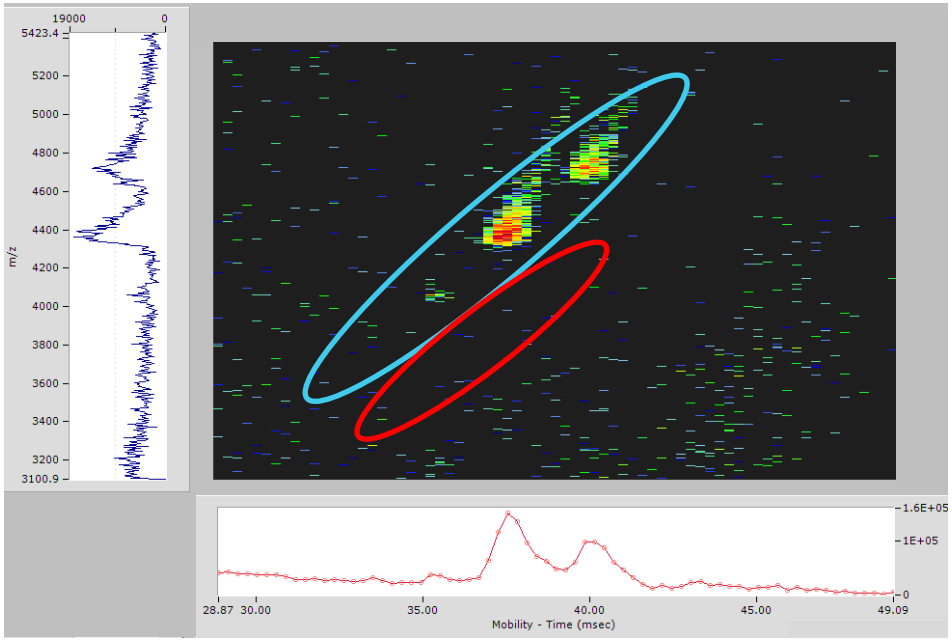


Protein complexes



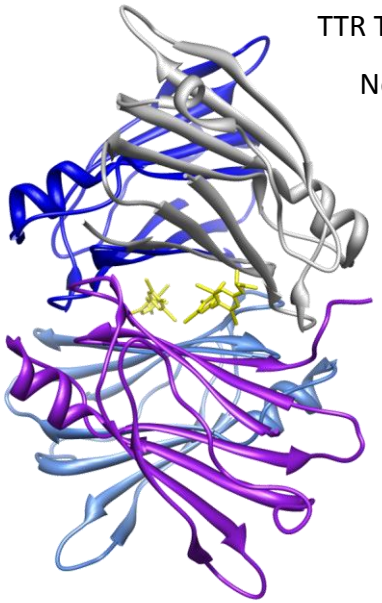
Transthyretin (TTR) Tetramer

Both compact and extended conformers



TTR Tetramer with 2 Diflunisal

No extended conformers



Drug stabilizes compact (solution phase) structure

Samples from Catherine Costello

Conclusions

IMS-MS:

- Separate small metabolite and lipid isomers
- Analyze samples short LC gradients or without LC for higher throughput
- Add further information to LC-MS analyses for additional and more confident identifications
- Measure kinetics of drugs and metabolites on protein complexes

Acknowledgements

- National Institute of Environmental Health Sciences of the NIH (R01ES022190)
- NIH: General Medical Sciences Proteomics Center at PNNL (2 P41 GM103493-11), National Institute of General Medical Sciences and National Cancer Institute
- PNNL Laboratory Directed Research and Development Program
- Environmental Molecular Sciences Laboratory